WHO/PHARM/86.529

ENGLISH ONLY

ACCELERATED STABILITY STUDIES OF WIDELY USED PHARMACEUTICAL SUBSTANCES UNDER SIMULATED TROPICAL CONDITIONS



WORLD HEALTH ORGANIZATION

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1. Introduction

Many pharmaceutical substances are known to deteriorate during distribution and storage particularly in hot, humid climates. Nonetheless, little precise information is available on the degradation characteristics of many long-established substances. More is known of the stability of recently introduced substances since relevant data must now be generated as a condition of registration to support proposed expiry dates. The results, however, are rarely published.

The World Health Organization has consequently commissioned a systematic survey of the stability of many widely used pharmaceutical substances. It has also developed simplified tests to detect or exclude gross degradation for the least stable substances.[1]

The conditions under which this survey were carried out were carefully standardized. Light was excluded, while temperature and humidity were adjusted to simulate tropical conditions. All substances were initially exposed for 30 days to air at 50 °C and 100 % humidity. If no degradation was demonstrable at this time, the temperature was raised to 70 °C for a further period of 3-7 days. The extent of degradation was assessed in most cases by thin-layer chromatography. A semi-quantitative method was used to determine the proportion of unaltered substance. The results of these tests have also been referred to in relevant monographs of the International Pharmacopoeia.[2]

"Standard degraded samples" were prepared under conditions described above that contained about 90 % of the original substance and 10 % of the degradation product. If necessary, final adjustments were made by mixing the degraded and original substances in appropriate proportion. The resulting mixture were then used to establish simple analytical procedures permitting

[1] WHO Technical Report Series, No. 645, 1979 (WHO Expert Committee on Specifications for Pharmaceutical Preparations, twenty-seventh report, section 5.2)

[2] The International Pharmacopoeia, third edition, volume 2, WHO, Geneva, 1981; volume 3 - in print.

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demonstration of the absence of gross degradation. These procedures are incorporated in WHO's basic tests for pharmaceutical substances.[1]

The study was initiated and coordinated by Dr M. Pesez, a member of the WHO Expert Advisory Panel on The International Pharmacopoeia and Pharmaceutical Preparations. The World Health Organization gratefully acknowledges the contribution of Dr M. Pesez and of Messrs Roussel-Uclaf SA, Romainville, France which generously offered facilities within their analytical laboratories to effect the work.

Acknowledgements are also due to Professor A.W.M. Indemans, Director of the Laboratory of the Dutch Pharmaceutical Society, The Hague, Netherlands, who kindly performed stability studies on some substances subject to narcotic regulations, and to Mr B. Ohrner, Director of the WHO Collaborating Centre for Chemical Reference Substances, Solna, Sweden who has corroborated many of the results obtained.[2]

2. Index of degradable substances

Priority was accorded to substances included within the WHO model list of essential drugs.[3] Altogether 110 of 296 substances tested were degraded under the conditions employed.

Acetylsalicylic acid
Aminophylline
Amitriptyline hydrochloride
Ammonium chloride
Amphotericin B
Ampicillin sodium
Ampicillin trihydrate
Antimony sodium tartrate
Ascorbic acid

Bacitracin
Bacitracin zinc
Benzathine benyzlpencillin
Benzylpencillin potassium
Benzylpencillin sodium
Bephenium hydroxynaphthoate

Calcium gluconate
Calcium para-aminosalicylate
Carbenicillin sodium
Cefalexin
Chloral hydrate
Chloramphenicol sodium succinate
Chlorphenamine hydrogen maleate
Chlorpromazine hydrochloride
Chlortetracycline hydrochloride
Cloxacillin sodium (monohydrate)
Coal tar

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1 Basic Tests for Pharmaceutical Substances, WHO, Geneva, 1986.

2 WHO Technical Report Series, No. 704, 1984 (WHO Expert Committee on Specifications for Pharmaceutical Preparations, twenty-ninth report).

3 WHO Technical Report Series, No. 722, 1985 (WHO Expert Committee on The Use of Essential Drugs).

Codeine phosphate
Colecalciferol
Cresol

Dapsone
Dexamethasone sodium phosphate
Dicloxacillin sodium (monohydrate)
Diethylcarbamazine dihydrogen citrate
Doxycycline hyclate

Emetine hydrochloride
Ephedrine
Ephedrine sulfate
Epinephrine
Epinephrine hydrogen tartrate
Ergocalciferol
Ergometrine hydrogen maleate
Ergotamine maleate
Ergotamine tartrate
Ethosuximide
Ethylmorphine hydrochloride

Ferrous sulfate
Fluphenazine decanoate
Fluphenazine hydrochloride
Formaldehyde solution

Gentamicin sulfate
Guanethidine sulfate

Hexylresorcinol
Hydralazine hydrochloride
Hydrocortisone sodium succinate
Hydroxocobalamin
Hyoscyamine sulfate

Imipramine hydrochloride
Ipecacuanha powder
Isoprenaline hydrochloride
Isoprenaline sulfate

Lidocaine hydrochloride

Melarsoprol
Mercuric oxide yellow
Metrifonate

Naloxone hydrochloride
Neomycin sulfate
Nystatin

Orciprenaline sulfate
Oxytetracyline hydrochloride

Paromomycin sulfate
Penicillamine
Pethidine hydrochloride
Phenobarbital sodium
Phenoxymethylpenicillin
Phenoxymethylpencillin calcium
Phenoxymethylpencillin potassium
Phentolamine mesilate
Phenylbutazone
Pilocarpine hydrochloride
Pilocarpine nitrate
Procainamide hydrochloride
Procaine benzylpencillin
Procaine hydrochloride
Procarbazine hydrochloride
Promazine hydrochloride
Promethazine hydrochloride
Pyridoxine hydrochloride

Quinine bisulfate
Quinine dihydrochloride

Retinol (Vitamin A)

Salbutamol sulfate
Senna leaf
Silver nitrate
Sodium calcium edetate
Sodium lactate
Sodium nitrite
Sodium para-aminosalicylate
Sodium stibogluconate
Sulfacetamide sodium
Sulfadiazine sodium
Sulfadimidine sodium
Suxamethonium chloride

Tetracaine hydrochloride
Tetracycline hydrochloride
Thiamine hydrochloride
Thiamine mononitrate
Thiopental sodium
Tolbutamide

Undecylenic acid

Warfarin sodium

3. Index of substances resistent to degradation

The following substances proved to be resistant to degradation under the conditions of the test. It is important to emphasise that light was excluded, since some of these substances may be readily degradable under the influence of light.

Acetazolamide
Acriflavinium chloride
Ajmaline
Allopurinol
Aluminium diacetate
Aluminium hydroxide
Amikacin
Amiloride hydrochloride
Aminocaproic acid
Aminophenazone
Amobarbital
Amodiaquine hydrochloride
Atropine sulfate
Azathioprine

Barbital
Barium sulfate
Beclometasone dipropionate
Benzocaine
Benzoic acid
Benzyl benzoate
Betamethasone
Betamethasone valerate
Bupivacaine hydrochloride
Busulfan

Caffeine
Carbamazepine
Cetrimide
Cetylpyridinium chloride
Charcoal, activated
Chloramphenicol
Chloramphenicol palmitate
Chlorhexidine diacetate
Chlorhexidine dihydrochloride
Chloroquine phosphate
Chlorothiazide
Chlorproguanil hydrochloride
Chlortalidone
Clofazimine
Clofibrate
Clomifene citrate
Codeine monohydrate
Colchicine
Cyanocobalamin

Dexamethasone
Dexamethasone acetate
Diazepam
Diazoxide
Dicoumarol
Diethylstilbestrol
Digitoxin
Digoxin
Diloxanide furoate
Dimercaprol
Diphenhydramine hydrochloride
Disodium edetate
Dopamine hydrochloride

Edrophonium chloride
Ephedrine hydrochloride
Erythromycin
Erythromycin ethylsuccinate
Erythromycin stearate
Ethambutol hydrochloride
Ethinylestradiol
Etynodiol diacetate

Fludrocortisone
Fludrocortisone acetate
Fluorescein sodium
Fluorouracil
Folic acid
Furosemide

Glibenclamide
Glucose
Griseofulvin

Haloperidol
*Halothane
Hexachlorophene
Hexobarbital
Homatropine hydrobromide
Hydrochlorothiazide
Hydrocortisone
Hydrocortisone acetate

Ibuprofen
Indometacin

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* Due to high volatilization of the substance at 50 °C, a degradation study is not feasible, though it justifies to apply stringent storage conditions.

Iodine
Isoniazid

Lactic acid
Lactose
Levodopa
Levonorgestrel
Lidocaine
Lindane
Lithium carbonate
Lobeline hydrochloride

Magnesium chloride
Magnesium oxide heavy
Magnesium oxide light
Mannitol
Mebendazole
Mepacrine hydrochloride
Meprobamate
Methyldopa
Methyltestosterone
Methylthiouracil
Metoclopramide hydrochloride
Metronidazole
Miconazole nitrate
Morphine hydrochloride

Neostigmine bromide
Neostigmine metilsulfate
Niclosamide
Nicotinamide
Nicotinic acid
Nifurtimox
Nikethamide
Niridazole
Nitrofurantoin
Norethisterone
Norethisterone acetate

Papaverine hydrochloride
Paracetamol
Pentamidine isetionate
Pentetrazol
Phenacetin
Phenobarbital
Phenolphthalein
Phentolamine hydrochloride
Phenytoin
Phenytoin sodium
Physostigmine salicylate
Phytomenadione
Piperazine adipate
Piperazine citrate
Potassium bromide
Potassium chloride
Potassium iodide
Prednisolone
Prednisolone acetate
Primaquine diphosphate
Probenecid
Progesterone
Proguanil hydrochloride
Propranolol hydrochloride
Propylthiouracil
Protionamide
Pyrazinamide
Pyridostigmine bromide
Pyrimethamine

Quinidine sulfate
Quinine dihydrochloride
Quinine sulfate

Reserpine
Riboflavin
Rifampicin

Salazosulfapyridine
Salicylic acid
Sodium bromide
Sodium chloride
Sodium cromoglicate
Sodium fluoride
Sodium hydrogen carbonate
Sodium metaperiodate
Sodium nitroprusside
Sodium salicylate
Sodium thiosulfate
Spironolactone
Streptomycin sulfate
Sucrose
Sulfadiazine
Sulfadimidine
Sulfadoxine
Sulfamethoxazole
Sulfamethoxypyridazine
Suramin sodium

Testosterone enantate
Testosterone propionate
Theobromine
Theophylline
Thioacetazone
Tiabendazole
Triamterene
Trihexyphenidyl hydrochloride
Trimethadione
Trimetoprim
Tubocurarine chloride

Zinc oxide
Zinc undecylenate

4. Stability experiments on degradable substances

ACETYLSALICYLIC ACID

Accelerated Degradation Experiment

Conditions

Procedure A: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 7 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of colourless crystals or a white, crystalline powder, remains unchanged after procedures A and B.

Gain of moisture

After procedures A and B: 4.5 %

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in ethanol 95 % - water (1:1).

Solvent system: heptane ..... 85 volumes
ethyl acetate ..... 10 volumes
acetic acid .....5 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is left in a chromatographic chamber containing some iodine crystals until spots appear.

Results: The spot obtained from the solution of the sample subjected to procedure A and the spot obtained from the reference solution of acetylsalicylic acid display the same chromatographic characteristics of migration and detection. No secondary spots are revealed.

The spot obtained from the solution of the sample subjected to procedure B and the spot obtained from the reference solution of salicylic acid display the same chromatographic characteristics of migration and detection. There is no longer a spot corresponding to acetylsalicylic acid.

Colorimetric determination

To 10 ml of a 0.1 % solution of the substance in water, add 1 ml of ethanol 95 %, 5 ml of 1 % ferric nitrate solution ($9H_2O$) in 1 % nitric acid, and dilute to 100 ml with water.

After procedure A: no violet coloration.
After procedure B: very intense violet coloration. By comparison with a reference concentration series of salicylic acid, the degradation was assessed at 75 %.

Conclusion

No decomposition is detected by thin-layer chromatography after procedure A, but after procedure B, the substance is completely degraded.

AMINOPHYLLINE

Accelerated Degradation Experiment

Conditions

Procedure A: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 4 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of white or slightly yellowish granules or a powder, changes after procedures A and B into a yellowish powder and the formation of needles is observed.

Gain of moisture

After procedure B: 80 %

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in acetone-water (1:1).

Solvent system: chloroform ..... 7 volumes
acetone ..... 3 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is left in a chromatographic chamber containing some iodine crystals until spots appear.

Results: The two spots obtained after procedures A and B correspond to theophylline and ethylenediamine. This method is useful to identify the substance, but not to estimate the degree of degradation.

Solubility

After procedures A and B, the solubility of the substance is modified. The insoluble part is identified as theophylline, and the degradation is assessed at 15 %.

Conclusion

Aminophylline is subject to decomposition under accelerated degradation conditions.

AMITRIPTYLINE HYDROCHLORIDE

Accelerated Degradation Experiment

Conditions

Procedure A: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 5 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of colourless crystals or a white or almost white powder, changes after procedures A and B into a viscous, amber-coloured solution.

Gain of moisture

After procedures A and B: 35 %

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in water.

Solvent system: n-butanol ..... 4 volumes
methanol ..... 6 volumes
acetic acid ..... 2 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is left in a chromatographic chamber containing some iodine crystals until spots appear.

Results: The spots obtained from the solutions of each of the samples subjected to procedures A and B and the spots obtained from the reference solution of amitriptyline hydrochloride display the same chromatographic characteristics of migration and detection. No secondary spots are revealed.

Conclusion

No decomposition is detected by thin-layer chromatography, but the appearance of the substance changes after procedures A and B.

AMMONIUM CHLORIDE

Accelerated Degradation Experiment

Conditions

Procedure A1: 1 day exposure at 50 °C and 100 % relative humidity.

Procedure A2: 10 days exposure at 50 °C and 100 % relative humidity.

Procedure A3: 17 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 2 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a white, crystalline powder, changes after procedures A and B into a colourless liquid.

Gain of moisture

After procedure A1: 5 %
After procedure A2: 40 %
After procedure A3: 65 %
After procedure B: 70 %

Titrimetric determination

Dissolve 0.1 g of each of the samples subjected to procedures A2, A3 and B in 20 ml of water. Add 5 ml of formaldehyde previously neutralized against phenolphthalein, and titrate with 0.1 N sodium hydroxide using the same indicator.

The reference substance of ammonium chloride contains 98 % of NH_4Cl.

After procedure A2: 60 %
After procedure A3: 35 %
After procedure B: 30 %

Conclusion

Ammonium chloride is very hygroscopic and is subject to fast decomposition under accelerated degradation conditions.

AMPHOTERICIN B

Accelerated Degradation Experiment

Conditions

Procedure A: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 5 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a yellow to orange powder, changes after procedure B into a yellow-ochre powder.

Gain of moisture

Before exposure to heat and humidity:	6 %
After procedure A:	27 %
After procedure B:	30 %

Chromatographic test

Adsorbent: Silica gel G

Solution applied to the plate: Substance dissolved in water.

Solvent system:

n-butanol	50 volumes
ethanol 95 %	25 volumes
concentrated ammonia	10 volumes
water	25 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is left in a chromatographic chamber containing some iodine crystals until spots appear.

Results: The spots obtained from the solutions of each of the samples subjected to procedures A and B and the spot obtained from the reference solution of amphotericin B display the same chromatographic characteristics of migration and detection, but the spots of the samples subjected to the test are slightly smaller and less intense.

Ultraviolet spectrophotometry

The absorption spectrum is recorded using a 0.001 % solution.

	405 nm	382 nm	363 nm
Before exposure to heat and humidity	1.094	0.902	0.539
After procedure A	0.736	0.607	0.362
After procedure B	0.630	0.570	0.345

Conclusion

Amphotericin B is subject to decomposition under accelerated degradation conditions.

AMPICILLIN SODIUM

Accelerated Degradation Experiment

Conditions

Procedure A: 20 hours exposure at 50 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a white or almost white powder, changes after procedure A into a hard, yellow mass.

Gain of moisture

Before exposure to heat and humidity:	1.6 %
After procedure A:	13 %

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in water.

Solvent system:

n-butanol	6 volumes
acetic acid	2 volumes
water	2 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is examined under ultraviolet light at a wavelength of maximum output at about 254 nm, and then left in a chromatographic chamber containing some iodine crystals until spots appear.

Results: The secondary spots obtained from the solution of the sample subjected to procedure A are more numerous than the corresponding spots obtained from the reference solution of ampicillin sodium.

Iodometric determination

After procedure A, the degradation was assessed at 73 %.

Ultraviolet spectrophotometry

Prepare a 0.1 % solution of the sample subjected to procedure A in water. Dilute 2 ml to 100 ml with buffered copper sulfate solution (dissolve 15.22 g of anhydrous disodium hydrogen phosphate in 536 ml of water, add sufficient aqueous 2.1 % citric acid solution until a pH of 5.15-5.25 is reached - about 464 ml. Mix 985 ml of this buffer solution to 15 ml of aqueous 0.393 % copper(II) sulfate solution). Heat 10 ml to 75 °C for 30 minutes, cool and measure the absorbance at about 320 nm, using the unheated buffered copper sulfate solution as a blank.

Before exposure to heat and humidity:	0.530
After procedure A:	0.175

Conclusion

Ampicillin sodium is subject to strong decomposition under accelerated degradation conditions.

AMPICILLIN TRIHYDRATE

Accelerated Degradation Experiment

Conditions

Procedure A: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 5 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a white or almost white, crystalline powder, changes after procedures A and B into a pale yellow to yellow powder.

Gain of moisture

None after procedures A and B.

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in water.

Solvent system: n-butanol ..... 6 volumes
acetic acid ..... 2 volumes
water ..... 2 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is left in a chromatographic chamber containing some iodine crystals until spots appear.

Results: The spot obtained from the solution of the sample subjected to procedure A and the spot obtained from the reference solution of ampicillin trihydrate display the same chromatographic characteristics of migration and detection. After procedure B, degradation occurs.

Conclusion

Ampicillin trihydrate is subject to decomposition under accelerated degradation conditions.

ANTIMONY SODIUM TARTRATE

Accelerated Degradation Experiment

Conditions

Procedure A: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 7 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of colourless, transparent scales or an almost white powder, changes after procedure A into a slightly transparent, white mass and after procedure B into a transparent, light yellow liquid.

Gain of moisture

Before exposure to heat and humidity:	7 %
After procedure A:	30 %
After procedure B:	55 %

Iodometric determination

The titration is performed in the presence of bicarbonate.

After procedures A and B, the degradation was assessed at 94 %.

Conclusion

Antimony sodium tartrate is subject to decomposition under accelerated degradation conditions.

ASCORBIC ACID

Accelerated Degradation Experiment

Conditions

Procedure A: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 8 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of colourless crystals or a white or almost white, crystalline and odourless powder, separates after procedure A into an upper phase, dark red-brown in colour, and a lower highly viscous phase, both having an odour resembling caramel. After procedure B both phases acquire a brownish black coloration.

Gain of moisture

After procedures A and B: 75 %.

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in water.

Solvent system: n-butanol ..... 6 volumes
acetic acid ..... 2 volumes
water ..... 2 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is left in a chromatographic chamber containing some iodine crystals until spots appear.

Results: The spot obtained from the solution of the sample subjected to procedure A and the spot obtained from the reference solution of ascorbic acid display the same chromatographic characteristics of migration and detection. After procedure B, degradation occurs.

Conclusion

No decomposition is detected by thin-layer chromatography after procedure A, but it is subject to severe decomposition after procedure B, as also seen from the change in the appearance of the substance and the gain of moisture.

BACITRACIN

Accelerated Degradation Experiment

Condition

Procedure A: 30 days exposure at 50 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a white or pale brownish yellow and odourless powder, changes after procedure A into a yellow-orange mass with a strong and disagreeable odour.

Gain of moisture

Before exposure to heat and humidity: 4 %
After procedure A: 46 %

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in water.

Solvent system: n-butanol ..... 6 volumes
acetic acid ..... 2 volumes
water ..... 2 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is left to dry in air and examined under ultraviolet light at a wavelength of maximum output at about 254 nm, then sprayed with triketohydrindene hydrate 0.25 % and heated at 100 °C until spots appear.

Results: The spots obtained from the solution of the sample subjected to procedure A do not show the same chromatographic characteristics of migration and detection as the spots obtained from a reference solution of bacitracin.

Conclusion

Bacitracin is subject to decomposition under accelerated degradation conditions.

BACITRACIN ZINC

Accelerated Degradation Experiment

Conditions

Procedure A : 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B : 30 days exposure at 50 °C and 100 % relative humidity followed by 5 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a white or pale brownish yellow powder, changes after procedure A into a hard yellow-golden compact mass turning to an orange colour after procedure B. During testing, a strong odour of sulfur dioxide can be detected.

Gain of moisture

After procedure A : 10 - 35 %
After procedure B : 7 - 10 %

Chromatographic test

Adsorbent : Silica gel 60 F-254

Solution applied to the plate : Substance dissolved in a mixture of methanol and water (1 : 1).

Solvent system : n-butanol.....50 volumes
ethanol 95 %.....15 volumes
water.....20 volumes
acetic acid.....15 volumes

Development conditions : The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection : The plate is examined under ultraviolet light at a wavelength of maximum output at about 254 nm, and then left in a chromatographic chamber containing some iodine crystals until spots appear. A second plate is sprayed with a 0.2 % solution of triketohydrindene hydrate in acetone, then heated at 100 - 105 °C for 15 minutes.

Results : The reference solution of bacitracin zinc shows numerous spots of different intensities. The solutions of each of the samples subjected to procedures A and B show diminished intensities of the main spots as well as additional spots.

Ultraviolet spectrophotometry

A reference solution in 0.05 M sulfuric acid shows 2 absorption maxima at 252 nm and 290 nm. The absorption values of the solutions of each of the samples subjected to procedures A and B and recalculated to the dried substance, remain similar at the wavelength of 252 nm, but increase significantly at 290 nm.

Differential scanning calorimetry

No significant change is observed after procedures A and B.

Conclusion

Bacitracin zinc is subject to decomposition under accelerated degradation conditions.

BENZATHINE BENZYLPENICILLIN

Accelerated Degradation Experiment

Conditions

Procedure A: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 5 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a white powder, changes after procedure A into a pale yellow powder and into pale yellow compact blocks after procedure B.

Gain of moisture

Before exposure to heat and humidity: less than 3 %
After procedures A and B: not more than 3 %

Chromatographic test

Adsorbent : Silica gel 60 F-254

Solution applied to the plate : Substance dissolved in methanol.

Solvent system : n-butanol.....50 volumes
water.....20 volumes
ethanol 95 %.....15 volumes
acetic acid.....15 volumes

Development conditions : The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection : The plate is examined under ultraviolet light at a wavelength of maximum output of about 254 nm, and then left in a chromatographic chamber containing some iodine crystals until spots appear.

Results : The reference solution of benzathine benzylpenicillin shows numerous spots of different intensities. No additional spots are observed from the solutions of each of the samples subjected to procedures A and B, and the intensities remain the same.

Ultraviolet spectrophotometry

A reference solution in methanol shows three absorption maxima at 251 nm, 257 nm and 263 nm. A maximum appears with time at a wavelength of 312 nm.

The characteristics of the absorption spectra of the solutions of the substances subjected to procedures A and B are modified. An additional maximum at 293 nm appears that increases between procedures A and B.

Infrared spectrophotometry

No change is observed after procedures A and B.

Differential scanning calorimetry

No significant change is observed after procedures A and B.

Conclusion

Benzathine benzylpenicillin is subject to decomposition under accelerated degradation conditions as determined by ultraviolet.

BENZYLPENICILLIN POTASSIUM

Accelerated Degradation Experiment

Conditions

Procedure A: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 5 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a white or almost white, crystalline powder, changes after procedures A and B into a viscous orange coloured liquid.

Gain of moisture

After procedures A and B: 38 %

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in water.

Solvent system: n-butanol ..... 6 volumes
acetic acid ..... 2 volumes
water ..... 2 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is left in a chromatographic chamber containing some iodine crystals until spots appear.

Results: The secondary spots obtained from the solution of the sample subjected to procedure A are more numerous and intense than the corresponding spots obtained from the reference solution of benzylpenicillin potassium. After procedure B, the spots are intensified.

Conclusion

Benzylpenicillin potassium is subject to decomposition under accelerated degradation conditions.

BENZYLPENICILLIN SODIUM

Accelerated Degradation Experiment

Conditions

Procedure A: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 5 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a white or almost white, crystalline powder, changes after procedures A and B into a viscous, orange-coloured liquid.

Gain of moisture

After procedures A and B: 35 %

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in water.

Solvent system: n-butanol ..... 6 volumes
acetic acid ..... 2 volumes
water ..... 2 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is left in a chromatographic chamber containing some iodine crystals until spots appear.

Results: The secondary spots obtained from the solution of the sample subjected to procedure A are more numerous and intense than the corresponding spots obtained from the reference solution of benzylpencillin sodium. After procedure B, the spots are intensified.

Conclusion

Benzylpencillin sodium is subject to decomposition under accelerated degradation conditions.

BEPHENIUM HYDROXYNAPHTHOATE

Accelerated Degradation Experiment

Conditions

Procedure A: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 5 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a yellow to greenish yellow, crystalline powder remains unchanged after procedure A, but acquires a dark beige colour with the apparition of small chestnut coloured dots after procedure B.

Gain of moisture

Before exposure to heat and humidity:	less than 0.1 %
After procedure A:	0.3 %
After procedure B:	less than 0.1 %

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in methanol.

Solvent system: n-butanol ..... 2 volumes
acetic acid ..... 1 volume
water ..... 1 volume

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The still humid plate is examined directly in daylight, then under ultraviolet light at a wavelength of maximum output at about 365 nm, and after drying at about 254 nm.

Results: The spots obtained from the solution of the sample subjected to procedure A and the spot obtained from the reference solution of bephenium hydroxynaphthoate display the same chromatographic characteristics of migration and detection. After procedure B, an additional spot is observed under ultraviolet light at a wavelength of 365 nm, showing a slightly green fluorescence and is located just below the principal spot at R_f 0.67.

Ultraviolet spectrophotometry

The absorption spectrum is recorded between 200 nm and 400 nm using a 0.0005 % solution of each of the samples subjected to procedures A and B in methanol.

Before exposure to heat and humidity: no change
After procedures A and B: no change

Solubility in methanol.

After procedure A: the solubility decreases.

Conclusion

Bephenium hydroxynaphthoate is subject to slight decomposition under accelerated degradation conditions.

CALCIUM GLUCONATE

Accelerated Degradation Experiment

Conditions

Procedure A: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 5 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of white, crystalline granules or a white, crystalline powder, changes after procedure B into a yellow mass.

Gain of moisture

Before exposure to heat and humidity:	0.7 %
After procedure A:	1.5 %

pH-value

Before exposure to heat and humidity:	6.45
After procedures A and B:	6.50

Specific optical rotation $[\alpha]_D^{20\ °C}$

Prepare a 3 % solution in water.

Before exposure to heat and humidity:	47 °
After procedure A:	46 °

Colorimetric determinations of organic impurities

Add 2 ml of concentrated sulfuric acid to 0.5 g of each of the samples subjected to procedures A and B: a yellow to brown coloration is produced.

Add 1 ml of 0.005 % of chromotrope 2B in concentrated sulfuric acid to each of the samples subjected to procedures A and B: the initial violet coloration changes to blue-green.

Conclusion

Calcium gluconate is subject to slight decomposition under accelerated degradation conditions.

CALCIUM PARA-AMINOSALICYLATE

Accelerated Degradation Experiment

Conditions

Procedure A: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 5 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a white or lightly-coloured, crystalline powder, changes after procedures A and B into a granular, dark chestnut coloured powder.

Gain of moisture

Before exposure to heat and humidity:	25 %
After procedure A:	39 %
After procedure B:	63 %

Chromatographic test

Adsorbent: Silica gel G

Solution applied to the plate: Substance dissolved in 2 N acetic acid.

Solvent system: methanol ..... 100 volumes
concentrated ammonia ..... 1.5 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is left in a chromatographic chamber containing some iodine crystals until spots appear.

Results: A spot appears on the starting line with the solution of the sample subjected to procedure A, and it is intensified with the solution of the sample subjected to procedure B. The main spot of the solutions of each of the samples subjected to procedures A and B decrease compared to the spot corresponding to calcium para-aminosalicylate.

Ultraviolet spectrophotometry

The absorbance is measured using 0.001 % solutions in methanol.

	301 nm	270 nm	231 nm
Before exposure to heat and humidity	0.537	0.706	0.640
After procedure A	0.442	0.558	0.510
After procedure B	0.376	0.493	0.448

Conclusion

Calcium para-aminosalicylate is subject to decomposition under accelerated degradation conditions.

CARBENICILLIN SODIUM

Accelerated Degradation Experiment

Conditions

Procedure A1: 8 hours exposure at 50 °C and 100 % relative humidity.

Procedure A2: 24 hours exposure at 50 °C and 100 % relative humidity.

Procedure A3: 2 1/2 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a white or almost white powder, changes after procedure A1 into a hard and sticky yellow mass, after procedure A2 into a yellow-orange gel, after procedures A3 and B into a yellow-orange liquid.

Gain of moisture

Before exposure to heat and humidity: 4.6 %
After procedure B: 9.3 %

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in water.

Solvent system: n-butanol ..... 6 volumes
acetic acid ..... 2 volumes
water ..... 2 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is examined under ultraviolet light at a wavelength of maximum output at about 254 nm, and then left in a chromatographic chamber containing some iodine crystals until spots appear.

Results: The secondary spots obtained from the solution of the sample subjected to procedure B are more numerous than the corresponding spots obtained from the reference solution of carbenicillin sodium.

Acidimetric titration

After procedure A2: 50 % degraded
After procedure B: 100 % degraded

Iodometric titration

After procedure A1: 30 % degraded
After procedure A2: 70 % degraded
After procedure A3: 90 % degraded
After procedure B: 93 % degraded

Conclusion

Carbenicillin sodium is subject to decomposition under accelerated degradation conditions.

CEFALEXIN

Accelerated Degradation Experiment

Conditions

Procedure A: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 5 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a white powder, changes after procedure A into an orange yellow solution and after procedure B into a hard, dark brown mass.

Gain of moisture

After procedures A and B: 58 %

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in a mixture composed of 5 volumes of methyl acetate, 3 volumes of acetone and 2 volumes of water to which 1 % of triethylamine is added.

Solvent system: 1 % of triethylamine in a mixture composed of:
methyl acetate ..... 5 volumes
acetone ..... 3 volumes
water ..... 1 volume

Development conditions: The solution is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is left in a chromatographic chamber containing some iodine crystals until spots appear.

Results: The spot obtained from the solution of the sample subjected to procedure A and the spot obtained from the reference solution of cefalexin display the same chromatographic characteristics of migration and detection. After procedure B, almost complete degradation occurs.

Conclusion

Cefalexin is subject to decomposition under accelerated degradation conditions.

CHLORAL HYDRATE

Accelerated Degradation Experiment

Conditions

Procedure A: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 4 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of transparent and colourless crystals, disappears after a few days due to complete sublimation.

Conclusion

Chloral hydrate disappears under accelerated degradation conditions.

CHLORAMPHENICOL SODIUM SUCCINATE

Accelerated Degradation Experiment

Conditions

Procedure A : 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B : 30 days exposure at 50 °C and 100 % relative humidity followed by 5 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a white to very pale yellow powder changes after procedure A into a pale yellow liquid and upon cooling turning into a heterogenous pale yellow liquid. After procedure B the colour turns to ochre yellow.

Gain of moisture

After procedures A and B : about 50 %

Chromatographic test

Adsorbent : Silica gel 60 F-254

Solution applied to the plate : Substance dissolved in methanol.

Solvent system : chloroform.....6 volumes
methanol.....4 volumes

Development conditions : The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection : The plate is examined under ultraviolet light at a wavelength of maximum output at about 254 nm, and then sprayed successively with a solution of titanium trichloride, and with dimethylaminobenzaldehyde or dimethylaminocinnamaldehyde dissolved in diluted hydrochloric acid. The plate is examined at about 365 nm.

Results : The reference solution of chloramphenicol sodium succinate shows two main spots corresponding to the two isomers of esterification in positions 1 and 3, as well as a weak spot corresponding to chloramphenicol. The solutions of each of the samples subjected to procedures A and B show diminished main spots and three additional spots. The spot corresponding to chloramphenicol increases first and diminishes gradually.

Ultraviolet spectrophotometry

Aqueous solutions of the samples subjected to procedures A and B show no modification of the absorption wavelength but the absorbances increase with time.

Gas-liquid chromatography

After procedures A and B, the peaks corresponding to the 2 isomers decrease considerably due to the hydrolysis of the ester; the formed chloramphenicol degrades and several degradation products appear as separate peaks.

Conclusion

Chloramphenicol sodium succinate is subject to decomposition under accelerated degradation conditions.

CHLORPHENAMINE HYDROGEN MALEATE

Accelerated Degradation Experiment

Conditions

Procedure A: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 5 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a white, crystalline powder separates after procedures A and B into two phases, white crystals in a red-brown coloured liquid.

Gain of moisture

After procedures A and B: 10 %

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in water.

Solvent system: n-butanol ..... 6 volumes
acetic acid ..... 2 volumes
water ..... 2 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is left in a chromatographic chamber containing some iodine crystals until spots appear.

Results: The spots obtained from the solutions of each of the samples subjected to procedures A and B and the spot obtained from the reference solution of chlorphenamine hydrogen maleate display the same chromatographic characteristics of migration and detection. The same characteristics are observed from the spot corresponding to maleic acid.

Conclusion

No decompositon is detected by thin-layer chromatography, but the appearance of the substance changes after procedures A and B.

CHLORPROMAZINE HYDROCHLORIDE

Accelerated Degradation Experiment

Conditions

Procedure A: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 8 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a white or slightly creamy white, crystalline powder, changes after procedures A and B into a viscous dark red-brown liquid.

Gain of moisture

After procedures A and B: 50 %

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in water.

Solvent system:

ammonia	5 volumes
toluene	60 volumes
dioxan	35 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is left in a chromatographic chamber containing some iodine crystals until spots appear.

Results: The reference substance of chlorpromazine hydrochloride reveals several secondary spots indicating small amounts of impurities. The spots obtained from the solutions of each of the samples subjected to procedures A and B and the spots obtained from the reference solution of chlorpromazine hydrochloride display the same chromatographic characteristics of migration and detection. The secondary spots remain unchanged, no more numerous nor intense. By comparison with a reference concentration series, the degradation was assessed at about 1 %.

Conclusion

No decomposition is detected by thin-layer chromatography, but the appearance of the substance changes after procedures A and B.

CHLORTETRACYCLINE HYDROCHLORIDE

Accelerated Degradation Experiment

Conditions

Procedure A: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 5 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of yellow crystals or a yellow, crystalline powder, changes after procedure A into a dark yellow powder and after procedure B into a dark brown powder.

Gain of moisture

None after procedures A and B.

Chromatographic test

Adsorbent: Cellulose with a fluorescent indicator, 0.1 mm in thickness, impregnated with 0.1 M disodium edetate and dried at 100 °C for 30 minutes.

Solution applied to the plate: Substance dissolved in methanol. After application, the plate is exposed to ammonia vapours for 15 minutes.

Solvent system:

n-butanol	10 volumes
pyridine	6 volumes
ethyl acetate	6 volumes
water	7 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is exposed to ammonia vapours and is examined under ultraviolet light at a wavelength of maximum output at about 365 nm.

Results: The reference substance of chlortetracycline hydrochloride reveals several spots. The spots obtained from the solutions of each of the samples subjected to procedures A and B and the spots obtained from the reference solution of chlortetracycline hydrochloride display the same chromatographic characteristics of migration and detection.

Conclusion

No decompositon is detected by thin-layer chromatography, but the appearance of the substance changes after procedures A and B.

CLOXACILLIN SODIUM (monohydrate)

Accelerated Degradation Experiment

Conditions

Procedure A1: 24 hours exposure at 50 °C and 100 % relative humidity.

Procedure A2: 48 hours exposure at 50 °C and 100 % relative humidity.

Procedure A3: 3 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 10 days exposure at 50 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a white, crystalline powder, changes after procedure A2 into a light yellow powder, after procedure A3 into a viscous yellow mass and after procedure B into a yellow-orange liquid which after cooling turns into a sticky, viscous paste.

Gain of moisture

Before exposure to heat and humidity: 5 %
After procedure B: 56 %

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in water.

Solvent system: n-butanol ..... 6 volumes
acetic acid ..... 2 volumes
water ..... 2 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is examined under ultraviolet light at a wavelength of maximum output at about 254 nm, and then left in a chromatographic chamber containing some iodine crystals until spots appear.

Results: The spots obtained from the solution of the sample subjected to procedure B compared to the spots obtained from the reference solution of cloxacillin sodium are more intense.

Acidimetric titration

After procedure A3: 22 % degraded
After procedure B: 67 % degraded

Iodometric titration

After procedure A1: 4 % degraded
After procedure A2: 16 % degraded
After procedure A3: 37 % degraded
After procedure B: 94 % degraded

Conclusion

Cloxacillin sodium is subject to decomposition under accelerated degradation conditions.

COAL TAR (washed)

Accelerated Degradation Experiment

Conditions

Procedure A : 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B : 30 days exposure at 50 °C and 100 % relative humidity followed by 5 days at 70 °C and 100 % relative humidity.

Appearance of the material

The material, originally in the form of a shiny black, viscous liquid, changes after procedures A and B into a more viscous liquid due to the elimination of its volatile components.

Gain of moisture

A small loss of weight is observed after procedures A and B.

Chromatographic test

Adsorbent : Silica gel 60 F-254

Solution applied to the plate : Substance dissolved in methanol.

Solvent system : light petroleum.....95 volumes
acetic acid..... 5 volumes

Development conditions : The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection : The plate is examined under ultraviolet light at a wavelength of maximum output of about 254 nm and then at 365 nm.

Results : The reference solution of coal tar shows a series of spots more or less visible depending on the detection method. No changes occur in the chromatogram obtained from the solutions of each of the samples subjected to procedures A and B.

Gas-liquid chromatography

The chromatogram shows a great number of peaks (about 30) with little modification after procedures A and B, the intensities of the peaks changing with time.

Conclusion

Coal tar is subject to decomposition under accelerated degradation conditions.

CODEINE PHOSPHATE

Accelerated Degradation Experiment

Conditions

Procedure A1: 10 days exposure at 50 °C and 100 % relative humidity.

Procedure A2: 20 days exposure at 50 °C and 100 % relative humidity.

Procedure A3: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B1: 30 days exposure at 50 °C and 100 % relative humidity followed by 4 days at 70 °C and 100 % relative humidity.

Procedure B2: 30 days exposure at 50 °C and 100 % relative humidity followed by 7 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of small colourless crystals or a white, crystalline powder, changes gradually after procedure A into a brown mass and after procedure B into a dark brown liquid.

Gain of moisture

Before exposure to heat and humidity:	5 %
After procedure A1:	16 %
After procedure A2:	30 %
After procedure A3:	42 %
After procedure B1:	45 %

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in methanol.

Solvent system:

diethylamine	1 volume
xylene	10 volumes
methanol	3 volumes
ethylmethylketone	10 volumes

Development condition: The solvent is allowed to migrate until the front reaches a line 10 cm from the starting line.

Detection: The plate is examined under ultraviolet light at a wavelength of maximum output at about 254 nm and then left in a chromatographic chamber containing some iodine crystals until spots appear.

Results: The spots obtained from the solution of the sample subjected to procedure A1 and the spots obtained from the reference solution of codeine phosphate display the same chromatographic characteristics. Some secondary spots are observed, one of which is on the starting line. After procedure B1, additional more and less mobile spots than the main spot are revealed, indicating a degradation of about 10 %.

High performance liquid chromatography

After procedures A and B: 9 % degraded, calculated with reference to the dried substance.

Conclusion

Codeine phosphate is subject to decomposition under accelerated degradation conditions.

COLECALCIFEROL

Accelerated Degradation Experiment

Conditions

Procedure A: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 4 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of colourless crystals or a white, crystalline powder, changes gradually after procedures A and B into a hard, reddish-brown mass.

Gain of moisture

After procedures A and B: 8 %

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in acetone.

Solvent system: chloroform

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is left in a chromatographic chamber containing some iodine crystals until spots appear.

Results: The spots obtained from the solution of the sample subjected to procedure A and the spots obtained from the reference solution of colecalciferol indicate a degradation of about 50 %. Several spots less mobile than the main spot and other more mobile spots are observed. After procedure B, the number of additional spots does not increase, but some are intensified.

Conclusion

Colecalciferol is subject to decomposition under accelerated degradation conditions.

CRESOL

Accelerated Degradation Experiment

Conditions

Procedure A1: 24 hours exposure at 50 °C and 100 % relative humidity.

Procedure A2: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 3 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The material, originally in the form of an almost colourless to pale brownish yellow liquid, changes after procedure A2 into a yellow to intense orange coloured liquid and after procedure B into a light brown liquid.

Gain of moisture

After procedures A2 and B: 9.2 %

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in ethanol 95 %.

Solvent system: chloroform ..... 95 volumes
methanol ..... 5 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is examined under ultraviolet light at a wavelength of maximum output at about 254 nm, then sprayed with molybdotungstophosphate reagent and exposed to ammonia vapours.

Results: Under ultraviolet light the spots obtained from the solutions of each of the samples subjected to procedures A2 and B and the spots obtained from the reference solution of cresol display the same chromatographic characteristics of migration and detection. After spraying no secondary spots are revealed with the solution from procedure A2. After procedure B, two traces are observed, one less mobile, the other more mobile than the main spot.

Ultraviolet spectrophotometry

1. The absorption spectrum is recorded between 200 nm and 400 nm using a 0.001 % solution of the samples subjected to procedures A2 and B in ethanol 95 %; a maximum is observed at about 280 nm.

2. The absorption spectrum is recorded between 200 nm and 400 nm using a 0.001 % solution of the samples subjected to procedures A2 and B in 0.1 N sodium hydroxide; maxima are observed at about 237 nm and 295 nm.

Results: Both spectra show no degradation.

Conclusion

No major decomposition is detected by thin-layer chromatography or by ultraviolet spectrophotometry, but the appearance changes after procedures A1, A2 and B.

DAPSONE

Accelerated Degradation Experiment

Conditions

Procedure A: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 4 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a white or creamy white, crystalline powder, changes after procedures A and B into a light cream-coloured powder.

Gain of moisture

After procedures A and B: 2.5 %

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in methanol.

Solvent system: chloroform ..... 7 volumes
acetone ..... 3 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is left in a chromatographic chamber containing some iodine crystals until spots appear, or the plate is sprayed with the following reagent until yellow spots appear: 1 g of p-dimethylaminobenzaldehyde, 30 ml of ethanol 95 %, 30 ml of concentrated hydrochloric acid and 180 ml of n-butanol.

Results: The spots obtained from the solutions of each of the samples subjected to procedures A and B and the spot obtained from the reference solution of dapsone display the same chromatographic characteristics of migration and detection. No secondary spots are revealed.

Conclusion

No decomposition is detected by thin-layer chromatography, but the appearance of the substance changes slightly after procedures A and B.

DEXAMETHASONE SODIUM PHOSPHATE

Accelerated Degradation Experiment

Conditions

Procedure A1: 10 days exposure at 50 °C and 100 % relative humidity.

Procedure A2: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 7 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a white or almost white, crystalline powder, changes after procedure A1 into a paste, its consistency resembling honey.

Gain of moisture

Before exposure to heat and humidity: 10 %
After procedures A2 and B: 40 %

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in methanol.

Solvent system: ethyl acetate ..... 5 volumes
acetone ..... 2 volumes
water ..... 1 volume
acetic acid ..... 2 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is either examined under ultraviolet light at a wavelength of maximum output at about 254 nm, and then left in a chromatographic chamber containing some iodine crystals until spots appear, or sprayed with a solution of 5 % phosphomolybdic acid in acetic acid containing 5 % sulfuric acid.

Results: The spots obtained from the solutions of each of the samples subjected to procedures A and B and the spots obtained from the reference solution of dexamethasone sodium phosphate display the same chromatographic characteristics of migration and detection. No additional secondary spot can be observed (the starting material shows two weak secondary spots).

Ultraviolet spectrophotometry

The absorbances are measured at a maximum wavelength at about 240.5 nm and calculated with reference to the dried substance.
After procedures A and B: no change is observed.

$A\,\frac{1\ \%}{1\ cm} = 271.$

Colorimetric determination of the carbonyl group in the A-ring

An ethanolic solution of the substance is treated with 4-aminophenazone in the presence of hydrochloric acid, and the absorbance is measured at 380 nm.

No change is observed.

Conclusion

No chemical modification is detected, but the substance is decomposed as seen from the important gain of moisture under accelerated degradation conditions.

DICLOXACILLIN SODIUM (monohydrate)

Accelerated Degradation Experiment

Conditions

Procedure A1: 24 hours exposure at 50 °C and 100 % relative humidity.

Procedure A2: 48 hours exposure at 50 °C and 100 % relative humidity.

Procedure A3: 3 days exposure at 50 °C and 100 % relative humidity.

Procedure A4: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 10 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a white or almost white, crystalline powder, changes after procedure A2 into a yellow powder, after procedure A3 into a viscous mass and finally after procedures A4 and B into a syrupy yellow-orange liquid which after cooling turns into a sticky, viscous paste.

Gain of moisture

Before exposure to heat and humidity:	4.3 %
After procedures A4 and B:	67 %

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in water.

Solvent system:

n-butanol	6 volumes
acetic acid	2 volumes
water	2 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is examined under ultraviolet light at a wavelength of maximum output at about 254 nm, and then left in a chromatographic chamber containing some iodine crystals until spots appear.

Results: The secondary spots obtained from the solutions of each of the samples subjected to procedures A4 and B are more numerous than the corresponding spots obtained from the reference solution of dicloxacillin sodium.

Acidimetric titration

After procedure A3:	20 % degraded
After procedures A4 and B:	60 % degraded

Iodometric titration

After procedure A1:	no degradation
After procedure A2:	7 % degraded
After procedure A3:	29 % degraded
After procedures A4 and B:	95 % degraded

Conclusion

Dicloxacillin sodium is subject to decomposition under accelerated degradation conditions.

DIETHYLCARBAMAZINE DIHYDROGEN CITRATE

Accelerated Degradation Experiment

Conditions

Procedure A1: 5 days exposure at 50 °C and 100 % relative humidity.

Procedure A2: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 7 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a white, crystalline powder, changes after procedures A1, A2 and B into a clear, colourless and slightly viscous liquid.

Gain of moisture

Before exposure to heat and humidity:	1 %
After procedures A2 and B:	81 %

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in water.

Solvent system:

ethanol 95 %	6 volumes
acetic acid	3 volumes
water	1 volume

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is left in a chromatographic chamber containing some iodine crystals until spots appear.

Results: The spots obtained from the solutions of each of the samples subjected to procedures A2 and B and the spot obtained from the reference solution of diethylcarbamazine dihydrogen citrate display the same chromatographic characteristics of migration and detection.

Conclusion

No decomposition is detected by thin-layer chromatography, but the appearance of the substance changes after procedures A1, A2 and B, due to a strong gain of moisture.

DOXYCYCLINE HYCLATE

Accelerated Degradation Experiment

Conditions

Procedure A: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 5 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a yellow, crystalline powder, darkens in colour after procedure A, and after procedure B changes into a brown-black powder.

Gain of moisture

Before exposure to heat and humidity: 2.5 %
After procedures A and B: 8 %

Chromatographic test

Adsorbent: Cellulose without fluorescent indicator, impregnated with 0.1 M disodium edetate and dried at 100 °C for 30 minutes.

Solution applied to the plate: Substance dissolved in methanol. After application the plate is exposed to ammonia vapours for 15 minutes.

Solvent system:

n-butanol	10 volumes
pyridine	6 volumes
ethyl acetate	6 volumes
water	7 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is exposed to ammonia vapours, and then examined under ultraviolet light at a wavelength of maximum output at about 365 nm.

Results: The spot obtained from the solution of the sample subjected to procedure A and the spot obtained from the reference solution of doxycycline hydrochloride display the same chromatographic characteristics of migration and detection. After procedure B intense secondary spots appear.

Ultraviolet spectrophotometry

The absorption spectrum is recorded between 200 nm and 400 nm using a 0.002 % solution of each of the samples subjected to procedures A and B in methanolic 0.1 N hydrochloric acid; a maximum is observed at about 349 nm.

After procedures A and B, no change is observed in the shape of the spectra.
After procedure A: no change
After procedure B: 30 % degraded.

Colorimetric determination

The content of doxycycline hydrochloride is determined with molybdotungstophosphate reagent in a medium containing carbonates, and the absorbance is measured at 750 nm.

After procedure A: no degradation
After procedure B: 30 % degraded

Conclusion

Doxycycline hydrochloride is subject to decomposition under accelerated degradation conditions.

EMETINE HYDROCHLORIDE

Accelerated Degradation Experiment

Conditions

Procedure A: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 3 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a white or very slightly yellow, crystalline powder, changes after procedure A into a creamy white powder.

Gain of moisture

After procedure A: 2.4 %

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in a mixture of methanol and water (8:2).

Solvent system:

cyclohexane	10 volumes
acetone	20 volumes
toluene	20 volumes
concentrated ammonia ...	1 volume

The solvent is mixed, decanted and after separation of the aqueous layer, the organic layer is used for the test.

Results: A series of spots are obtained from the solution of the sample subjected to procedure A compared to the spot obtained from the reference solution of emetine hydrochloride.

High performance liquid chromatography

After procedure A: 13 % degraded

Ultraviolet spectrophotometry

The spectrum of a 0.1 % solution of the sample subjected to procedure A in methanol confirms degradation.

Conclusion

Emetine hydrochloride is subject to decomposition under accelerated degradation conditions.

EPHEDRINE

Accelerated Degradation Experiment

Conditions

Procedure A: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 5 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of colourless crystals or a white, crystalline powder, odourless or with a slight aromatic odour, changes after procedures A and B into a viscous, colourless and transparent liquid with a slight odour.

Gain of moisture

Before exposure to heat and humidity:	4.5 %
After procedure A:	10 %
After procedure B:	10 %

Chromatographic test

Adsorbent: Silica gel G

Solution applied to the plate: Substance dissolved in methanol.

Solvent system:

n-butanol	6 volumes
acetic acid	2 volumes
water	2 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is either left in a chromatographic chamber containing iodine crystals until spots appear or sprayed with a 0.2 % solution of triketohydrindene hydrate in acetone and heated at 100 °C.

Results: The spots obtained from the solutions of each of the samples subjected to procedures A and B and the spots obtained from the reference solution of ephedrine do not show the same chromatographic characteristics of migration and detection. The main spot of the reference solution migrates less far than the ones of the solutions of the samples subjected to the test, and for these, an additional spot remains at the point of application.

Ultraviolet spectrophotometry

The absorbance is measured using a 0.05 % solution of the samples in 0.1 N sulfuric acid.

	263 nm	257 nm	251 nm
Before exposure to heat and humidity	0.433	0.570	0.478
After procedure A	0.158	0.646	0.572
After procedure B	0.562	0.961	0.884

Colorimetric determination

The colorimetric method is based on the formation of picrates extracted into chloroform.

Decomposition is observed.

Conclusion

Ephedrine is subject to decomposition under accelerated degradation conditions.

EPHEDRINE SULFATE

Accelerated Degradation Experiment

Conditions

Procedure A: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 7 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of colourless crystals or a white, crystalline powder, changes after procedures A and B into deliquescent crystals.

Gain of moisture

Before exposure to heat and humidity: 1 %
After procedure A: 5 %
After procedure B: 15 %

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in methanol.

Solvent system: dioxan ..... 15 volumes
ammonia 3 N ..... 2 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is sprayed with a solution containing 0.1 % of triketohydrindene hydrate in acetone and heated at 100 °C for 3 minutes.

Results: The spots obtained from the solutions of each of the samples subjected to procedures A and B and the spots obtained from the reference solution of ephedrine sulfate display the same chromatographic characteristics of migration and detection. No secondary spots are revealed.

Ultraviolet spectrophotometry

The aborbances of ethanolic solutions are measured at about 253 nm, 259 nm and 275 nm.

After procedures A and B: no significant change.

Acidimetric determination

After extraction of the ephedrine base with chloroform in alkaline medium.

After procedures A and B: no change.

Solubility

The rate of solubility of the samples subjected to procedures A and B is slower than for the untreated substance.

Conclusion

No chemical decomposition is detected, but the gain of moisture and the solubility change after procedures A and B indicate degradation.

EPINEPHRINE

Accelerated Degradation Experiment

Conditions

Procedure A: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 4 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a white or almost white, microcrystalline powder, changes rapidly after procedure A into an orange brown powder and after procedure B into a viscous dark red-brown liquid.

Gain of moisture

After procedures A and B: 63 %

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in 0.25 N hydrochloric acid.

Solvent system: n-butanol ..... 6 volumes
acetic acid ..... 2 volumes
water ..... 2 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is left in a chromatographic chamber containing some iodine crystals until spots appear.

Results: The chromatogram obtained from the solution of the sample subjected to procedure A in comparison to the chromatogram obtained from the reference solution of epinephrine displays tailing on either side of the main spot indicating a degradation of about 50 %.

Conclusion

Epinephrine is subject to strong decomposition under accelerated degradation conditions.

EPINEPHRINE HYDROGEN TARTRATE

Accelerated Degradation Experiment

Conditions

Procedure A1: 1 day exposure at 50 °C and 100 % relative humidity.

Procedure A2: 5 days exposure at 50 °C and 100 % relative humidity.

Procedure A3: 10 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a white to greyish white, crystalline powder, changes gradually after procedure A1 into a greyish compact mass, after 2 days into a viscous, yellowish liquid, becoming after procedures A2 and A3 brown and finally black.

Gain of moisture

After procedure A1: 10-20 %
After procedure A2: 25-45 %
After procedure A3: 30-50 %

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in a 0.2 % solution of sodium metabisulfite.

Solvent system: n-butanol ..... 6 volumes
acetic acid ..... 2 volumes
water ..... 2 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is examined under ultraviolet light at a wavelength of maximum output at about 254 nm, and then examined in white light after exposure to iodine vapours.

Results: Several spots are obtained from the solutions of each of the samples subjected to procedures A1, A2, A3 and B, being more or less mobile than the main spot, when compared to the corresponding spots of the reference solution of epinephrine hydrogen tartrate.

Colorimetric determination

Prepare 0.002 % solutions of the samples subjected to procedures A1 and A2 in a 0.5 % solution of sodium metabisulfite. Similarly prepare a reference solution of epinephrine hydrogen tartrate.

Transfer to separate test-tubes 1 ml of the solutions to be examined and 1 ml of the reference solution. To each tube add 2 ml of 0.5 % sodium metabisulfite solution, 2 ml of water, 1 ml of 0.1 % thiosemicarbazide solution and 0.5 ml of 1 N sodium hydroxide. Allow to stand at room temperature for 45 minutes and measure the absorbances at about 480 nm.

After procedure A1: 69 %, not including the moisture content of 10-20 %, representing a degradation of more than 10 %.

Conclusion

Epinephrine hydrogen tartrate is subject to strong decomposition under accelerated degradation conditions.

ERGOCALCIFEROL

Accelerated Degradation Experiment

Conditions

Procedure A: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 4 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of colourless or slightly yellowish crystals or a white or slightly yellowish, crystalline powder, changes after procedure A into a slightly yellowish powder and after procedure B into a hard orange-brown mass.

Gain of moisture

After procedures A and B: 1 %

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in chloroform.

Solvent system: chloroform

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is left in a chromatographic chamber containing some iodine crystals until spots appear.

Results: The spots obtained from the solution of the sample subjected to procedure A and the spots obtained from the reference solution of ergocalciferol display the same chromatographic characteristics of migration and detection, though some of the spots are slightly more

intense. The degradation was assessed at less than 5 %. After procedure B, additional spots, some more mobile and some less mobile than the main spot, are revealed, indicating gross degradation. The degradation was assessed at about 40 %.

Conclusion

Ergocalciferol is subject to slight decomposition after procedure A, but strong decomposition is observed after procedure B.

ERGOMETRINE HYDROGEN MALEATE

Accelerated Degradation Experiment

Conditions

Procedure A: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 3 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a white or faintly yellow, crystalline powder, changes after procedure A into a greyish anthracite coloured powder and after procedure B into a black powder.

Gain of moisture

After procedure A: 11 %

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in a mixture of methanol and water (8:2).

Solvent system: 2-propanol ..... 4 volumes
acetone ..... 2 volumes
concentrated ammonia .... 4 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is examined under ultraviolet light at a wavelength of maximum output at about 254 nm and 365 nm, and then left in a chromatographic chamber containing some iodine crystals until spots appear.

Results: The spots obtained from the solution of the sample subjected to procedure A and the spots obtained from the reference solution of ergometrine hydrogen maleate display the same chromatographic characteristics of migration and detection. After procedure B, two additional spots are obtained and some tailing.

Conclusion

Ergometrine hydrogen maleate is subject to decomposition under accelerated degradation conditions.

ERGOTAMINE MALEATE

Accelerated Degradation Experiment

Conditions

Procedure A: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 4 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a white powder, turns black after a few days.

Gain of moisture

After procedure A: 15 %
After procedure B: 24 %

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in water.

Solvent system: methanol ..... 20 volumes
chloroform ..... 80 volumes
concentrated ammonia ... 0.2 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is examined under ultraviolet light at a wavelength of maximum output at about 254 nm, and then left in a chromatographic chamber containing some iodine crystals until spots appear.

Results: After procedure A, the spot obtained from the solution of the sample is slightly less intense than the spot obtained from the reference solution of ergotamine maleate. In addition, a secondary spot appears. After
procedure B, the chromatogram remains unchanged.

Ultraviolet spectrophotometry

The absorbance is measured at about 311 nm using a 0.002 % solution of the samples in ethanol 95 %. After procedures A and B, a decrease of absorbance of about 25 % is noted.

Colorimetric determination

The absorbance is measured at about 555 nm using a solution of the samples to which dimethylaminobenzaldehyde has been added.

After procedure A, a decrease of absorbance is noted corresponding to a decomposition of about 30 %.

Conclusion

Ergotamine maleate is subject to decomposition under accelerated degradation conditions.

ERGOTAMINE TARTRATE

Accelerated Degradation Experiment

Conditions

Procedure A: 10 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of colourless crystals or a greyish white to yellowish white, crystalline powder, changes after procedure A into a greyish powder and after procedure B into a black powder.

Gain of moisture

Before exposure to heat and humidity:	4.6 %
After procedure A:	7.8 %
After procedure B:	8.8 %

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in water containing 1 % of tartaric acid.

Solvent system: methanol ..... 100 volumes
concentrated ammonia ... 1.5 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is examined under ultraviolet light at a wavelength of maximum output at about 254 nm, and then left in a chromatographic chamber containing some iodine crystals until spots appear.

Results: The spots obtained from the solution of the sample subjected to procedure A and the spots obtained from the reference solution of ergotamine tartrate display the same chromatographic characteristics of migration and detection. No secondary spots are revealed. After procedure B, the R_f value of the main spot is modified with a decrease in intensity.

Ultraviolet spectrophotometry

The absorbance is measured at about 239 nm using an aqueous solution containing 1 % of tartaric acid.

After procedure A: 4 % degraded
After procedure B: 21 % degraded, no change is noticed in the shape of the spectrum.

Colorimetric determination

The absorbance is measured at about 400 nm using a solution to which picric acid has been added.

After procedure A: 3 % degraded
After procedure B: 35 % degraded

Conclusion

Ergotamine tartrate is subject to decomposition under accelerated degradation conditions.

ETHOSUXIMIDE

Accelerated Degradation Experiment

Conditions

Procedure A1: 10 days exposure at 50 °C and 100 % relative humidity.

Procedure A2: 20 days exposure at 50 °C and 100 % relative humidity.

Procedure A3: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 5 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a white or almost white powder or waxy solid, changes after procedure A1 into a syrupy liquid, after procedure A2 into a syrupy liquid containing small suspended droplets, after procedure A3 into two phases forming an emulsion and after procedure B into a homogenous liquid.

Gain of moisture

After procedure A3: 24.5 %
After procedure B: 15 %

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in water.

Solvent system: chloroform ..... 8 volumes
acetone ..... 2 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is left in a chromatographic chamber containing some iodine crystals until spots appear.

Results: The spots obtained from the solutions of each of the samples subjected to procedures A1, A2, A3, and B, and the spots obtained from the reference solution of ethosuximide display the same chromatographic characteristics of migration and detection. No secondary spots are revealed.

Ultraviolet spectrophotometry

The absorbance is measured at about 248 nm using a solution in ethanol 95 %.

After procedures A and B: no degradation is observed, taking the content of moisture into consideration.

Conclusion

No decomposition is detected by thin-layer chromatography, but the appearance of the substance changes after procedures A and B.

ETHYLMORPHINE HYDROCHLORIDE

Accelerated Degradation Experiment

Conditions

Procedure A1: 10 days exposure at 50 °C and 100 % relative humidity.

Procedure A2: 20 days exposure at 50 °C and 100 % relative humidity.

Procedure A3: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B1: 30 days exposure at 50 °C and 100 % relative humidity followed by 4 days at 70 °C and 100 % relative humidity.

Procedure B2: 30 days exposure at 50 °C and 100 % relative humidity followed by 7 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a white, crystalline powder changes gradually after procedure A into a slightly brownish, crystalline powder and after procedure B into a brownish mass.

Gain of moisture

Before exposure to heat and humidity:	9 %
After procedure A1:	9 %
After procedure A2:	9 %
After procedure A3:	9 %
After procedure B2:	60 %

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in methanol.

Solvent system:

diethylamine	1 volume
xylene	10 volumes
methanol	3 volumes
ethylmethylketone	10 volumes

Development condition: The solvent is allowed to migrate until the front reaches a line 10 cm from the starting line.

Detection: The plate is examined under ultraviolet light at a wavelength of maximum output at about 254 nm and then left in a chromatographic chamber containing some iodine crystals until spots appear.

Results: The spots obtained from the solution of the sample subjected to procedure A1 and the spots obtained from the reference solution of ethylmorphine hydrochloride display the same chromatographic characteristics. After procedure B2 additional more or less mobile spots than the main spot are revealed, indicating degradation.

High performance liquid chromatography

Before exposure to heat and humidity:	8 % degraded
After procedures A and B:	59 % degraded

Conclusion

Ethylmorphine hydrochloride is subject to decomposition under accelerated degradation conditions.

FERROUS SULFATE

Accelerated Degradation Experiment

Conditions

Procedure A: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 4 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of pale blue green prisms or a pale green, crystalline powder, efflorescent in air, changes after a few days into a yellow-chestnut coloured suspension.

Gain of moisture

After procedure A: 50 %
After procedure B: 60 %

Colorimetric determination

Determination of the ferric ion.

After procedures A and B: the quantity of oxidized product increases with time, it reaches 70 %.

Conclusion

Ferrous sulfate is subject to decomposition under accelerated degradation conditions.

FLUPHENAZINE DECANOATE

Accelerated Degradation Experiment

Conditions

Procedure A: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 7 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a pale yellow, viscous liquid or a yellow, crystalline, oily solid, changes after procedure B into a chestnut coloured solid.

Gain of moisture

After procedures A and B: 4 %

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in ethanol 95 %.

Solvent system: cyclohexane ..... 30 volumes
acetone .....20 volumes
concentrated ammonia ... 1 volume

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is left in a chromatographic chamber containing some iodine crystals until spots appear.

Results: The intensity of the main spots obtained from the solution of the sample subjected to procedure A are decreasing in comparison to the reference solution of fluphenazine decanoate, whereas the intensity of the less mobile secondary spots are increasing. After procedures A and B, the intensity of the secondary spots have decreased even more. The degradation was estimated at about 80 %.

Ultraviolet spectrophotometry and colorimetric determination

The spectra of the solutions of each of the samples subjected to procedures A and B remain unchanged.

Conclusion

Fluphenazine decanoate is subject to decomposition under accelerated degradation conditions.

FLUPHENAZINE HYDROCHLORIDE

Accelerated Degradation Experiment

Conditions

Procedure A: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 7 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a white or almost white, crystalline powder, changes after procedure A into a grey mass.

Gain of moisture

After procedure A: 34 %

Chromatographic test

Adsorbent: Aluminium oxide F-254

Solution applied to the plate: Substance dissolved in ethanol 95 %.

Solvent system: methanol ..... 100 volumes
concentrated ammonia ..... 1.5 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is left in a chromatographic chamber containing some iodine crystals until spots appear.

Results: The spots obtained from the solution of the sample subjected to procedure A and the spots obtained from the reference solution of fluphenazine hydrochloride display the same chromatographic characteristics of migration, but the intensity of the spots of the sample subjected to the test diminish.

Ultraviolet spectrophotometry and colorimetric determination

After procedure A, the UV measurement at 260 nm and the colorimetric determination after formation of a picrate at 400 nm , indicate a degradation of about 10 %.

Conclusion

Fluphenazine hydrochloride is subject to decomposition under accelerated degradation conditions.

FORMALDEHYDE SOLUTION

Accelerated Degradation Experiment

Conditions

Procedure A: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 4 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a colourless or almost colourless liquid, remains unchanged after procedures A and B.

Colorimetric determination

The method is based on the reaction with oxalyl dihydrazide and cupric acetate. Measure the absorbance at about 620 nm.

After procedures A and B an almost complete decomposition is observed.

Conclusion

Formaldehyde solution is subject to decomposition under accelerated degradation conditions.

GENTAMICIN SULFATE

Accelerated Degradation Experiment

Conditions

Procedure A: 30 days exposure at 50 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a white to cream-coloured powder, changes after procedure A into a dark brown liquid.

Gain of moisture

Before exposure to heat and humidity: 15 %
After procedure A: 55 %

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in water.

Solvent system: chloroform ..... 5 volumes
methanol ..... 4 volumes
concentrated ammonia ..... 2 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is left to dry in air and then examined under ultraviolet light at a wavelength of maximum output at about 254 nm. The plate is then sprayed with 0.25 % of triketohydrindene hydrate in acetone and heated at 100 °C until spots appear.

Results: The spot obtained from the solution of the sample subjected to procedure A and the spot obtained from the reference solution of gentamicin sulfate display the same chromatographic characteristics of migration, but the spot of the sample subjected to the test is less intense. Furthermore, an almost invisible secondary spot appears with the reference solution.

Ultraviolet spectrophotometry

The absorption spectrum is recorded using a 0.05 % solution in water.

After procedure A an absorbance at 320 nm is observed, which is not present in the reference solution.

Conclusion

Gentamicin sulfate is subject to decomposition under accelerated degradation conditions.

GUANETHIDINE SULFATE

Accelerated Degradation Experiment

Conditions

Procedure A1: 10 days exposure at 50 °C and 100 % relative humidity.

Procedure A2: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 3 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a light yellow powder, changes after procedure A2 into a yellow to buff-coloured powder and after procedure B into a curdy, yellow to buff-coloured powder.

Gain of moisture

After procedure A2: 6 %
After procedure B: 9 %

Chromatographic test

Adsorbent: Neutral aluminium oxide

Solution applied to the plate: Substance dissolved in a mixture of methanol and water (8:2).

Solvent system: 2-propanol ..... 4 volumes
acetone ..... 2 volumes
concentrated ammonia ..... 2 volumes

Development condition: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is left in a chromatographic chamber containing some iodine crystals until spots appear.

Results: The spots obtained from the solutions of each of the samples subjected to procedures A1 and A2, and the spots obtained from the reference solution of guanethidine sulfate display the same chromatographic characteristics of migration and detection. After procedure B, two additional spots are revealed, one on the starting line, the other at R_f 0.3.

Quantitative determination by colorimetry

Using sodium ferricyanide potassium ferricyanide, the estimated degradation is about 13 %.

Conclusion

Guanethidine sulfate is subject to decomposition under accelerated degradation conditions.

HEXYLRESORCINOL

Accelerated Degradation Experiment

Conditions

Procedure A: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 5 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of fine white or very slightly pink needles, changes after procedure A into a liquid that solidifies on cooling, resulting to a dark pink mass, and after procedure B into a liquid that solidifies on cooling, giving a light red-brown mass.

Gain of moisture

After procedure B: 6 %

Melting point

Before exposure to heat and humidity: 62 °C
After procedure B: 60 °C

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in methanol.

Solvent system: chloroform ..... 8 volumes
ethanol 95 %..... 2 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is left in a chromatographic chamber containing some iodine crystals until spots appear.

Results: The spots obtained from the solution of each of the samples subjected to procedures A and B and the spots obtained from the reference solution of hexylresorcinol display the same chromatographic characteristics of migration and detection.

Conclusion

No decompositon is detected by thin-layer chromatography, but after procedures A and B, the appearance of the substance changes.

HYDRALAZINE HYDROCHLORIDE

Accelerated Degradation Experiment

Conditions

Procedure A: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 5 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a white or almost white, crystalline powder, changes after procedure A into a rose beige, slightly agglomerated and sticky powder, and after procedure B into a darker beige powder containing chestnut-coloured particles.

Gain of moisture

Before exposure to heat and humidity:	0.6 %
After procedure A:	3 %
After procedure B:	1.2 %

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in water.

Solvent system:

chloroform	70 volumes
methanol	30 volumes
concentrated ammonia	1 volume

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: After drying the plate at about 35 °C for 2 hours, it is left in a chromatographic chamber containing some iodine crystals until spots appear.

Results: The spots obtained from the solutions of each of the samples subjected to procedures A and B, and the spots obtained from the reference solution of hydralazine hydrochloride display the same chromatographic characteristics of migration and detection. No additional secondary spots are revealed.

Colour of solution in water

After procedures A and B: dark yellow.

Ultraviolet spectrophotometry

After procedures A and B: no change is observed in the spectra.

pH value

Prepare a 1 % aqueous solution, allowing to stabilize for 1 hour before measurement.

Before exposure to heat and humidity:	4.8
After procedures A and B:	4.1

Conclusion

No decompositon is detected by thin-layer chromatography and by UV, but the appearance of the substance and the coloration of the solution in water after procedures A and B indicate degradation.

HYDROCORTISONE SODIUM SUCCINATE

Accelerated Degradation Experiment

Conditions

Procedure A: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 7 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a white or almost white, crystalline powder or amorphous solid changes after procedure A into a liquid, then into a compact yellow mass turning to orange after procedure B.

Gain of moisture

Before exposure to heat and humidity:	8.5 %
After procedure A:	30 %
After procedure B:	36 %

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in a mixture of chloroform-methanol (1:1).

Solvent system:

dichloromethane	77 volumes
methanol	8 volumes
ether	15 volumes
water	1.2 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is examined under ultraviolet light at a wavelength of maximum output at about 254 nm, then left in a chromatographic chamber containing some iodine crystals until spots appear. A second plate is sprayed with a 5 % solution of phosphomolybdic acid in acetic acid containing 5 % of sulfuric acid.

Results: A reference solution of hydrocortisone sodium succinate shows a main spot and several weak secondary spots; the spot at R_f 0.18 corresponds to hydrocortisone, another at R_f 0.58. A solution of the sample subjected to procedure A shows that the spot at R_f 0.18 decreases, whereas the spot at R_f 0.58 increases. After procedure B, the change is even more significant.

Ultraviolet spectrophotometry

The absorbances of solutions of samples subjected to the test measured at about 241 nm and calculated with reference to the dried substance remain unchanged.

Colorimetric determination of the carbonyl-group in the A-ring

A solution in ethanol 95 % is treated with 4-aminophenazone in the presence of hydrochloric acid and measured at 380 nm.

No significant change is detected.

Colorimetric determination of the ketone chain

The product is treated with 4-nitrosodimethylaniline in alkaline medium. The N,N-dimethyl-p-phenylenediamine produced is detected with phenol in presence of ferricyanide ion and measured at 650 nm.

No significant change is detected.

Conclusion

Hydrocortisone sodium succinate is subject to decomposition under accelerated degradation conditions.

HYDROXOCOBALAMIN

Accelerated Degradation Experiment

Conditions

Procedure A: 10, 20 then 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 7 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of dark red crystals or a red, crystalline powder, changes after procedures A and B into a dark red compact mass.

Gain of moisture

Before exposure to heat and humidity:	18 %
After procedure A:	27 %
After procedure B:	43 %

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in a mixture of ethanol 95 %-water (1:1).

Solvent system:

n-butanol	50 volumes
ethanol 95 %	25 volumes
concentrated ammonia	10 volumes
water	25 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: Direct observation of the spots in daylight.

Results: A reference solution of hydroxocobalamin shows a main spot and a very weak secondary spot. A solution of the sample subjected to procedure A shows that the secondary spot increases, whereas the main spot decreases. After procedure B, the degradation represents more than 50 % inversion of the size of the spots.

Ultraviolet spectrophotometry

The absorbances are measured at about 350 nm.

A solution of the samples subjected to the test shows a regular decrease in absorbance during the course of procedure A and after 30 days the degradation reaches 50 %. After procedure B a similar result is obtained.

Conclusion

Hydroxocobalamin is subject to strong decomposition under accelerated degradation conditions.

HYOSCYAMINE SULFATE

Accelerated Degradation Experiment

Conditions

Procedure A1: 10 days exposure at 50 °C and 100 % relative humidity.

Procedure A2: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 7 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a white, crystalline powder changes after procedure A1 into a yellow liquid.

Gain of moisture

After procedure A1: 25 %

Chromatographic test

Adsorbent: Aluminium oxide F-254

Solution applied to the plate: Substance dissolved in ethanol 95 %.

Solvent system: methanol ..... 100 volumes
concentrated ammonia ..... 3 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is left in a chromatographic chamber containing some iodine crystals until spots appear.

Results: The spots obtained from the solution of the sample subjected to procedure A1 and the spots obtained from the reference solution of hyoscyamine sulfate display the same chromatographic characteristics of migration and detection.

Ultraviolet spectrophotometry and colorimetric determination

After procedure A1, the UV measurement and the colorimetric determination after formation of a picrate at 400 nm , indicate a degradation of slightly larger than 10 %.

Conclusion

Hyoscyamine sulfate is subject to decomposition under accelerated degradation conditions.

IMIPRAMINE HYDROCHLORIDE

Accelerated Degradation Experiment

Conditions

Procedure A: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 5 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a white, crystalline powder, changes after procedure A into a grey powder and after procedure B into a liquid that solidifies on cooling into a light brown mass.

Melting point

Before exposure to heat and humidity:	175 °C
After procedure A:	172 °C
After procedure B:	162 °C

Gain of moisture

After procedures A and B: 10 %

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in methanol.

Solvent system: n-butanol ..... 2 volumes
acetic acid ..... 1 volume
water ..... 1 volume

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is examined under ultraviolet light at a wavelength of maximum output at about 254 nm and 365 nm respectively, then examined in white light after exposure to iodine vapours.

Results: The spots obtained from the solution of the sample subjected to procedure A and the spots obtained from the reference solution of imipramine hydrochloride display the same chromatographic characteristics of migration and detection. After procedure B, four additional spots are obtained under UV light at about 365 nm.

Ultraviolet spectrophotometry

After procedure A: no change in the spectrum.

Conclusion

Imipramine hydrochloride is subject to decompositon under accelerated degradation conditions.

IPECACUANHA POWDER

Accelerated Degradation Experiment

Conditions

Procedure A : 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B : 30 days exposure at 50 °C and 100 % relative humidity followed by 5 days at 70 °C and 100 % relative humidity.

Appearance of the material

The material, originally in the form of a pale grey to brown-yellowish powder changes after procedures A and B into a dark brown paste.

Gain of moisture

After procedures A and B : 30 - 45 %

Chromatographic test

Adsorbent : Silica gel 60 F-254

Solution applied to the plate : Chloroform layer after extraction of the material with ammonia.

Solvent system : toluene.....35 volumes
benzene.....35 volumes
ethyl acetate.....20 volumes
diethylamine.....10 volumes
methanol..... 2 volumes

Development condition : The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection : The plate is examined under ultraviolet light at a wavelength of maximum output at about 254 nm and then at 365 nm. After spraying with a solution of potassium iodobismuthate the plate is examined at 365 nm.

Results : The reference solution of ipecacuanha shows numerous spots of different intensities depending on the detection method. The spots obtained from the solutions of each of the samples subjected to procedures A and B correspond in number and position to the reference solution, but the intensities are considerably and irregularly modified.

Gas-liquid chromatography

The chromatograms of solutions of samples subjected to procedures A and B are modified significantly, the size of the main peaks, corresponding to emetine and cephaeline, decrease, whereas the unidentified secondary peaks increase considerably in intensity.

Conclusion

Ipecacuanha is subject to decomposition under accelerated degradation conditions.

ISOPRENALINE HYDROCHLORIDE

Accelerated Degradation Experiment

Conditions

Procedure A: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 5 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a white or almost white, crystalline powder, changes after procedure A into a light yellow powder and after procedure B into a light brown solution containing crystals of the same colour.

Gain of moisture

After procedures A and B: 65 %

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in methanol.

Solvent system: n-butanol ..... 6 volumes
acetic acid ..... 2 volumes
water ..... 2 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is left in a chromatographic chamber containing some iodine crystals until spots appear.

Results: The spots obtained from the solution of the sample subjected to procedure A and the spots obtained from the reference solution of isoprenaline hydrochloride display the same chromatographic characteristics of migration and detection. After procedure B, tailing is observed below the main spot indicating a degradation of 5 %.

Ultraviolet and infrared spectrophotometry

After procedures A and B: no change in the spectra.

Conclusion

Isoprenaline hydrochloride is subject to decomposition under accelerated degradation conditions.

ISOPRENALINE SULFATE

Accelerated Degradation Experiment

Conditions

Procedure A: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 4 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a white or almost white, crystalline powder, changes after procedure A into a pasty, light brown powder and after procedure B into a dark red-brown solution.

Gain of moisture

After procedures A and B: 33 %

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in water.

Solvent system: n-butanol ..... 6 volumes
acetic acid ..... 2 volumes
water ..... 2 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is left in a chromatographic chamber containing some iodine crystals until spots appear.

Results: The chromatogram obtained from the solution of the sample subjected to procedure A in comparison to the chromatogram obtained from the reference solution of isoprenaline sulfate displays tailing below the main spot indicating a degradation of less than 10 %. After procedure B, the substance displays the same chromatographic characteristics as after procedure A. The degradation remains unchanged.

Conclusion

Isoprenaline sulfate is subject to decomposition under accelerated degradation conditions.

LIDOCAINE HYDROCHLORIDE

Accelerated Degradation Experiment

Conditions

Procedure A: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 5 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a white, crystalline powder remains unchanged after procedure A, but after procedure B changes into a brownish liquid that solidifies on cooling and after some time results into a reddish brown mass.

Gain of moisture

After procedure B: 3 %

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in methanol.

Solvent system: n-butanol ..... 6 volumes
acetic acid ..... 2 volumes
water ..... 2 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is left in a chromatographic chamber containing some iodine crystals until spots appear.

Results: The spots obtained from the solutions of each of the samples subjected to procedures A and B and the spot obtained from the reference solution of lidocaine hydrochloride display the same chromatographic characteristics of migration and detection.

Conclusion

No decomposition is detected by thin-layer chromatography, but the appearance of the substance changes after procedure B.

MELARSOPROL

Accelerated Degradation Experiment

Conditions

Procedure A1: 10 days exposure at 50 °C and 100 % relative humidity.

Procedure A2: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 5 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of fine, white granules changes after procedure A1 into a slightly yellow coloured powder with some agglomeration on the surface and having a light perceptible odour of mercaptone. No further change is observed after procedures A2 and B.

Gain of moisture

After procedures A and B: practically no change.

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in pyridine/methanol (1:4). Use freshly prepared solutions.

Solvent system: n-butanol ..... 60 volumes
ethanol 95 %.....40 volumes
concentrated ammonia ..... 5 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: After drying the plate until the odour of ammonia has disappeared, it is left in a chromatographic chamber containing some iodine crystals until spots appear.

Results: The spots obtained from the solution of the sample subjected to procedure A1 and the spots obtained from the reference solution of melarsoprol display the same chromatographic characteristics of migration and detection, except of the spot remaining on the starting line being much more intense. The results remain the same after procedures A2 and B.

Solution in pyridine/methanol

After procedure A1 a strong opalescence is observed in the solution even after addition of methanol, whereas the reference solution remains clear.

Ultraviolet spectrophotometry

Prepare a 0.5 % solution of the sample subjected to procedure A1 in 1 N hydrochloric acid/methanol (1:9); a slight precipitate is formed, whereas the reference solution of melarsoprol remains clear. The spectrum of the filtered test solution is similar to that of the reference solution with a maximum at 285 nm and a shoulder at 255 nm.

Conclusion

Melarsoprol is subject to decomposition after procedure A1, which does not seem to increase after procedures A2 and B. It is not possible to estimate the percentage of degradation.

MERCURIC OXIDE YELLOW

Accelerated Degradation Experiment

Conditions

Procedure A: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 4 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a fine, yellow-orange powder, changes after procedure A into a brown coloured powder.

Gain of moisture

After procedure A: 3 %
After procedure B: 6 %

Chemical determination

Titration of the mercuric ion with thiocyanate in the presence of ferric ammonium sulfate.

After procedures A and B: rapid decomposition, reaching 90 %.

Conclusion

Mercuric oxide yellow is subject to decomposition under accelerated degradation conditions.

METRIFONATE

Accelerated Degradation Experiment

Conditions

Procedure A1: 10 days exposure at 50 °C and 100 % relative humidity.

Procedure A2: 20 days exposure at 50 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a white or yellowish white, crystalline powder, changes after procedure A1 into a colourless liquid that recrystallizes at room temperature, and after procedure A2 recrystallization does not reoccur, not even at a temperature of 0 °C.

Gain of moisture

Before exposure to heat and humidity: about 0.2 %
After procedure A1: 46 %
After procedure A2: 67.5 %

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in water.

Solvent system: chloroform ..... 6 volumes
acetone ..... 4 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: Prepare the following spraying solutions:

Solution I - 0.05 N silver nitrate in methanol.

Solution II - 30 % aqueous solution of formaldehyde.

Solution III - 2 N potassium hydroxide in methanol.

Solution IV - A mixture of equal volumes of 40 % nitric acid with an aqueous 3 % hydrogen peroxide solution.

After drying the plate, spray it in the following manner: Spray with solution I, allow to rest at room temperature for 30 minutes; spray with solution II, allow to rest at room temperature for 30 minutes; spray with solution III, heat at 100 °C for 30 minutes; spray with solution IV, allow to rest in the dark overnight, then expose it to daylight for a few hours. Violet spots of the chlorine derivatives appear on a white background.

Results: The spots obtained from the solution of the sample subjected to procedure A1 is weaker by a factor of 2 with an important spot on the starting line when compared with the reference solution of metrifonate. The main spot is intensified after procedure A2.

Determination of ionisable chlorine by argentometry

Before exposure to heat and humidity: None.
After procedure A1: 2.8 % calculated with reference to the dried substance.
After procedure A2: 5.1 % calculated with reference to the dried substance.

Determination of free aldehydes

Absorbance of a reference solution of metrifonate containing 100 µg/ml: 0.075

Absorbance of a solution of the substance subjected to procedure A1 containing 100 µg/ml: 0.62

Absorbance of a solution of the substance subjected to procedure A2 containing 10 µg/ml: 0.22

Conclusion

Metrifonate is subject to decomposition under accelerated degradation conditions.

NALOXONE HYDROCHLORIDE

Accelerated Degradation Experiment

Conditions

Procedure A1: 10 days exposure at 50 °C and 100 % relative humidity.

Procedure A2: 20 days exposure at 50 °C and 100 % relative humidity.

Procedure A3: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 5 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a white or almost white powder, changes after procedure A3 into a beige coloured powder and after procedure B into a dark beige powder.

Gain of moisture

The substance contains 2 moles of water, equivalent to 9 % H_2O.

After procedure A3: 23.6 %
After procedure B: 9 %, representing the initial 2 moles of water.

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in water.

Solvent system:

n-butanol	100 volumes
methanol	10 volumes
concentrated ammonia	2 volumes

Development conditions: The development is performed with protection from light. The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is dried in the dark until the odour of ammonia has disappeared, the plate is then left in a chromatographic chamber containing some iodine crystals until spots appear.

Results: The spots obtained from the solutions of each of the samples subjected to procedures A3 and B, and the spots obtained from the reference solution of naloxone hydrochloride display the same chromatographic characteristics of migration and detection, a main spot at R_f 0.72 and a weak spot at R_f 0.85. After procedure B an additional very weak spot is revealed on the starting line.

Specific optical rotation

Before exposure to heat and humidity $[\alpha]_D^{20\,°C}$: -178.9 °

After procedures A3 and B determined $[\alpha]_D^{20\,°C}$: -179.3 °

Conclusion

Naloxone hydrochloride is subject to slight decomposition and the degradation was assessed at less than 5 %.

NEOMYCIN SULFATE

Accelerated Degradation Experiment

Conditions

Procedure A: 8 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure C: 30 days exposure at 50 °C and dry atmosphere.

Appearance of the substance

The substance, originally in the form of a white or yellowish white and odourless or almost odourless powder, changes after procedure A into a dark brown liquid with a strong odour. After procedure C there is no further change.

Gain of moisture

Before exposure to heat and humidity:	2.6 %
After procedure B:	37 %

Ultraviolet spectrophotometry

The spectrum of an aqueous solution of the sample subjected to procedure B shows a deformation of the curve with an absorption maximum at about 310 nm. After procedure C, no further change is observed .

Microbiological activity

The results are expressed with reference to the dried substance.

Before exposure to heat and humidity:	667 U/mg
After procedure B:	625 U/mg.

Conclusion

Neomycin sulfate is subject to decomposition under accelerated degradation conditions.

NYSTATIN

Accelerated Degradation Experiment

Conditions

Procedure A: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 5 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a yellow to light brown powder, changes after procedure A into a yellow powder and after procedure B into a dark orange-yellow powder.

Gain of moisture

None after procedures A and B.

Tests on the sample subjected to accelerated degradation

Thin-layer chromatography can not be carried out since the solubility of the substance in methanol is only 4 mg/ml. This solubility decreases after procedures A and B, and an insoluble residue is left. No solvent can be found to dissolve this residue in order to carry out the test.

Through separation and weighing the insoluble residue in dimethylformamide the degradation after procedure A is assessed at 20 % and after procedure B at 50 %.

The degradation is confirmed by microbiological measurement of the soluble portion of the sample subjected to procedures A and B. The activity obtained is 3000 U/mg, compared to 4405 U/mg for the reference substance of nystatin.

Conclusion

Nystatin is subject to decomposition under accelerated degradation conditions.

ORCIPRENALINE SULFATE

Accelerated Degradation Experiment

Conditions

Procedure A: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 7 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of fine, white crystals, changes after procedure A into an amber-coloured liquid.

Gain of moisture

After procedure A: 39 %

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in 0.01 N sulfuric acid.

Solvent system: methanol ..... 100 volumes
concentrated ammonia ..... 1.5 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is left in a chromatographic chamber containing some iodine crystals until spots appear.

Results: The spots obtained from the solution of the sample subjected to procedure A and the spots obtained from the reference solution of orciprenaline sulfate display the same chromatographic characteristics of migration and detection. No additional spots are obtained.

Ultraviolet spectrophotometry and colorimetric determination

After procedure A, the UV absorbance at 276 nm and the colorimetric determination through the reaction with chloranil, indicate a degradation of about 35 %.

Conclusion

Orciprenaline sulfate is subject to decomposition under accelerated degradation conditions.

OXYTETRACYCLINE HYDROCHLORIDE

Accelerated Degradation Experiment

Conditions

Procedure A: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 5 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a yellow, crystalline powder changes after procedure A into a light red-brown powder and after procedure B into a black powder.

Gain of moisture

After procedures A and B: 10 %

Chromatographic test

Adsorbent: Cellulose with a fluorescent indicator, 0.1 mm in thickness, impregnated with 0.1 M disodium edetate and dried at 100 °C for 30 minutes.

Solution applied to the plate: Substance dissolved in methanol. After application the plate is exposed to ammonia vapours for 15 minutes.

Solvent system:

n-butanol	10 volumes
pyridine	6 volumes
ethyl acetate	6 volumes
water	7 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is exposed to ammonia vapours and is examined under ultraviolet light at a wavelength of maximum output at about 365 nm.

Results: The secondary spots obtained from the solution of the sample subjected to procedure A compared to the spots obtained from the reference solution of oxytetracycline hydrochloride are more intense. The degradation was assessed at about 2 %. After procedure B, additional spots appear and the secondary spots are more intense, whereas the main spot corresponding to oxytetracycline hydrochloride is much weaker.

Ultraviolet spectrophotometry

The spectrum of the solution of the sample subjected to procedure A confirms degradation.

Conclusion

Oxytetracycline hydrochloride is subject to decomposition under accelerated degradation conditions.

PAROMOMYCIN SULFATE

Accelerated Degradation Experiment

Conditions

Procedure A: 30 days exposure at 50 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a creamy white to light yellow powder, changes after procedure A into a dark brown liquid.

Gain of moisture

Before exposure to heat and humidity:	6 %
After procedure A:	50 %

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in water.

Solvent system: chloroform ..... 5 volumes
methanol ..... 4 volumes
concentrated ammonia ..... 2 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is left in the air to dry, and then examined under ultraviolet light at a wavelength of maximum output at about 254 nm. The plate is then sprayed with 0.25 % triketohydrindene hydrate in acetone and heated at 100 °C until spots appear.

Results: The spot obtained from the solution of the sample subjected to procedure A and the spot obtained from the reference solution of paromomycin sulfate display the same chromatographic characteristics of migration and detection, though after procedure A the spot is less intense and in addition, a secondary spot is revealed of much stronger intensity than the one obtained from the reference solution.

Ultraviolet spectrophotometry

The absorption spectrum is recorded using a 0.05 % solution in water.

After procedure A, an absorption maximum at about 320 nm is observed that is not present in the reference solution.

Conclusion

Paromomycin sulfate is subject to decomposition under accelerated degradation conditions.

PENICILLAMINE

Accelerated Degradation Experiment

Conditions

Procedure A: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 2 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a white or almost white, crystalline powder, changes after procedures A and B into agglomerates.

Gain of moisture

After procedures A and B: 20 %

Chromatographic test

Adsorbent: Silica gel F-254

Appearance of the substance

The substance, originally in the form of white, crystalline granules or a white, crystalline powder, changes after procedures A2 and A3 into a liquid with a white crust, after homogenization a white suspension is obtained.

Gain of moisture

After procedure A3: 54.8 %

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in ethanol 50 %.

Solvent system: toluene ..... 7 volumes
dioxan ..... 3 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is examined under ultraviolet light at a wavelength of maximum output at about 254 nm.

Results: After procedure A1, an additional weak spot is observed which is less mobile than the main spot. After procedure A2, the main spot is decreased and the additional spot is estimated at a degradation of about 20 %. After procedure A3, a decrease of even more importance is observed of the main spot and the significant increase in the additional spot is estimated at a degradation of about 50 %.

Ultraviolet spectrophotometry

The spectrum recorded between 200 nm and 400 nm, using 0.001 % solutions of each of the samples subjected to procedures A1, A2 and A3 in 0.1 N sodium hydroxide, show an absorption maximum at about 255 nm.

After procedure A1: 5 % degraded
After procedure A2: 25 % degraded
After procedure A3: 54 % degraded

Conclusion

Phenobarbital sodium is subject to decomposition under accelerated degradation conditions.

PHENOXYMETHYLPENICILLIN

Accelerated Degradation Experiment

Conditions

Procedure A: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 5 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a white, fine crystalline powder, changes after procedure A into a solid, pale yellow mass and after procedure B into a solid, orange-coloured mass.

Gain of moisture

After procedures A and B: 75 %

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in ethanol 95 %.

Solvent system: n-butanol ..... 6 volumes
acetic acid ..... 2 volumes
water ..... 2 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is left in a chromatographic chamber containing some iodine crystals until spots appear.

Results: The spots obtained from the solutions of each of the samples subjected to procedures A and B, compared to the spots obtained from the reference solution of phenoxymethylpenicillin are more intense and more numerous.

Conclusion

Phenoxymethylpenicillin is subject to decomposition under accelerated degradation conditions.

PHENOXYMETHYLPENICILLIN CALCIUM

Accelerated Degradation Experiment

Conditions

Procedure A1: 10 days exposure at 50 °C and 100 % relative humidity.

Procedure A2: 20 days exposure at 50 °C and 100 % relative humidity.

Procedure A3: 30 days exposure at 50 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a white, fine crystalline powder changes after procedure A1 into a yellowish white powder with yellow spots, after procedure A2 into a light yellow mass containing some air bubbles, the consistency of which is gum-like at 50 °C and hard at room temperature and after procedure A3 into a thick yellow syrup.

Gain of moisture

Before exposure to heat and humidity for the substance containing 2 moles of water: 4.8 %
After procedure A3: 26 %

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in water.

Solvent system: n-butanol ..... 6 volumes
acetic acid ..... 2 volumes
water ..... 2 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is left in a chromatographic chamber containing some iodine crystals until spots appear.

Results: After procedure A1, the main spot is slightly deformed and a more intense tailing is observed by comparison with the spots obtained from the reference solution of phenoxymethylpenicillin calcium. After procedure A2, the intensity of the main spot has decreased indicating a degradation of about 50 %. Several more mobile and less mobile spots than the main spots are also observed. After procedure A3, the intensity of the main spot has decreased even more, indicating a degradation of more than 50 % but the chromatogram resembling otherwise the one after procedure A2.

Conclusion

Phenoxymethylpenicillin calcium is subject to decomposition under accelerated degradation conditions, the decomposition being estimated at about 50 % after procedure A2.

PHENOXYMETHYLPENICILLIN POTASSIUM

Accelerated Degradation Experiment

Conditions

Procedure A1: 10 days exposure at 50 °C and 100 % relative humidity.

Procedure A2: 20 days exposure at 50 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a white or almost white, crystalline powder, odourless or with a faint chracteristic odour, changes after procedure A1 into a syrupy yellow-orange liquid, that solidifies into a sticky, viscous paste with a characteristic odour.

Gain of moisture

Before exposure to heat and humidity: 2 %
After procedure A1: 39 %

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in methanol.

Solvent system: n-butanol ..... 6 volumes
acetic acid ..... 2 volumes
water ..... 2 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is left in a chromatographic chamber containing some iodine crystals until spots appear.

Results: The spots obtained from the solutions of each of the samples subjected to procedures A1 and A2, compared to the spots obtained from the reference solution of phenoxymethylpenicillin potassium are more numerous.

Ultraviolet spectrophotometry

The spectrum of a 0.025 % solution of the samples subjected to procedures A1 and A2 in methanol show maxima at 263 nm, 268 nm and 274 nm. No degradation is observed.

Iodometric determination

After procedure A1: 30 % degraded
After procedure A2: 90 % degraded

Conclusion

Phenoxymethylpenicillin potassium is subject to decomposition under accelerated degradation conditions.

PHENTOLAMINE MESILATE

Accelerated Degradation Experiment

Conditions

Procedure A1: 20 days exposure at 50 °C and 100 % relative humidity.

Procedure A2: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 7 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a white, crystalline powder, changes after procedure A1 into a dark brown liquid.

Gain of moisture

After procedure A1: 35 %

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in methanol.

Solvent system: n-butanol ..... 6 volumes
acetic acid ..... 2 volumes
water ..... 2 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is left in a chromatographic chamber containing some iodine crystals until spots appear.

Results: After procedure A1, the intensity of the main spots decreases slightly by comparison with the spots obtained from the reference solution of phentolamine mesilate. An additional less mobile spot is also observed.

Ultraviolet spectrophotometry

The absorbance of a solution of the sample subjected to procedure A1 indicates a degradation of 17.5 %.

Conclusion

Phentolamine mesilate is subject to decomposition under accelerated degradation conditions.

PHENYLBUTAZONE

Accelerated Degradation Experiment

Conditions

Procedure A: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 4 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a fine white or creamy white, crystalline powder, remains unchanged after procedure A, but acquires a heterogeneous white to yellowish colour after procedure B.

Gain of moisture

After procedures A and B: 0.05 %

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in methanol.

Solvent system: cyclohexane ..... 80 volumes
acetone ..... 20 volumes
acetic acid ..... 2 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is left in a chromatographic chamber containing some iodine crystals until spots appear.

Results: The spots obtained from the solution of the sample subjected to procedure A and the spots obtained from the reference solution of phenylbutazone display the same chromatographic characteristics of migration and detection. No secondary spots are revealed. After procedure B, three additional spots are obtained that are less mobile than the main spot.

Titrimetric determination

The degradation after procedure B was determined by acidimetry in acetone and assessed at 6.6 %.

Conclusion

No decomposition is detected after procedure A, but phenylbutazone is subject to decomposition after procedure B.

PILOCARPINE HYDROCHLORIDE

Accelerated Degradation Experiment

Conditions

Procedure A: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 3 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of colourless crystals or a white, crystalline powder, changes after procedures A and B into a clear and colourless liquid.

Gain of moisture

After procedure A: 50 %
After procedure B: 60 %

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in a mixture of methanol and water (8:2).

Solvent system: acetone ..... 30 volumes
toluene ..... 20 volumes
concentrated ammonia ..... 2 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is left in a chromatographic chamber containing some iodine crystals until spots appear.

Results: The spots obtained from the solutions of each of the samples subjected to procedures A and B and the spot obtained from the reference solution of pilocarpine hydrochloride display the same chromatographic characteristics of migration and detection.

High performance liquid chromatography

After procedure B an impurity is detected.

Colorimetric determination

Reaction with hydroxylamine and ferric chloride.

After procedure A: no degradation
After procedure B: 25 % degraded

Conclusion

Pilocarpine hydrochloride is subject to decomposition under accelerated degradation conditions.

PILOCARPINE NITRATE

Accelerated Degradation Experiment

Conditions

Procedure A: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 3 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of colourless crystals or a white, crystalline powder, changes after procedure A into a solid mass and after procedure B into a paste but remaining white.

Gain of moisture

After procedure A: 1 %
After procedure B: 64 %

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in a mixture of methanol and water (8:2).

Solvent system: acetone ..... 30 volumes
toluene ..... 20 volumes
concentrated ammonia .. 2 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is left in a chromatographic chamber containing some iodine crystals until spots appear.

Results: The spots obtained from the solutions of each of the samples subjected to procedures A and B and the spot obtained from the reference solution of pilocarpine nitrate display the same chromatographic characteristics of migration and detection.

High performance liquid chromatography

After procedure B an impurity is detected.

Colorimetric determination

Reaction with hydroxylamine and ferric chloride.

After procedure A: no degradation
After procedure B: 25 % degraded

Conclusion

Pilocarpine nitrate is subject to decomposition under accelerated degradation conditions.

PROCAINAMIDE HYDROCHLORIDE

Accelerated Degradation Experiment

Conditions

Procedure A: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 7 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a white to yellowish white, crystalline powder, changes after procedures A and B into a light chestnut coloured solution.

Gain of moisture

Before exposure to heat and humidity: 0.5 %
After procedures A and B: 34 %

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in methanol.

Solvent system: methanol ..... 100 volumes
concentrated ammonia ..... 1.5 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is left in a chromatographic chamber containing some iodine crystals until spots appear.

Results: The spots obtained from the solutions of each of the samples subjected to procedures A and B and the spot obtained from the reference solution of procainamide hydrochloride display the same chromatographic characteristics of migration and detection.

Ultraviolet spectrophotometry

The spectrum recorded between 200 nm and 350 nm of 0.001 % solutions of the samples under test in 0.01 N sodium hydroxide, shows an absorption maximum at about 272 nm. No degradation is detected.

Colorimetric determination

The method is based on a diazo-reaction with sodium nitrite, sulfamic acid and naphthylethylenediamine hydrochloride. Absorption maximum: about 550 nm.

After procedures A and B: no degradation is detected.

Conclusion

No decompositon is detected by thin-layer chromatography, ultraviolet spectrophotometry and colorimetric determination, but the appearance of the substance changes after procedures A and B.

PROCAINE BENZYLPENICILLIN

Accelerated Degradation Experiment

Conditions

Procedure A: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C in a dry atmosphere.

Appearance of the substance

The substance, originally in the form of a white or almost white, crystalline powder, changes after procedure A into yellowish agglomerates. After procedure B the appearance of the substance remains unchanged.

Gain of moisture

Before exposure to heat and humidity:	4.7 %
After procedure A:	5.3 %
After procedure B:	4.7 %

Solubility and colour of solution

Dissolve 0.1 g of the substance in 10 ml of water.

Before exposure to heat and humidity:	colourless
After procedure A:	yellow
After procedure B:	colourless

Melting point

Before exposure to heat and humidity:	119 °C
After procedure A:	117.7 °C
After procedure B:	119°C

Iodometric determination

Before exposure to heat and humidity:	1030 U/mg
After procedure A:	994 U/mg
After procedure B:	1008 U/mg

Microbiological activity (turbidimetric method)

Before exposure to heat and humidity:	1065 U/mg
After procedure A:	1005 U/mg
After procedure B:	1055 U/mg

Ultraviolet and infrared spectrophotometry

No changes are observed in the spectra.

Conclusion

Procaine benzylpenicillin is slightly decomposed under accelerated degradation conditions.

PROCAINE HYDROCHLORIDE

Accelerated Degradation Experiment

Conditions

Procedure A: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 5 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of colourless crystals or a white, crystalline powder, changes after procedure A into a slightly yellow-coloured powder and after procedure B into a liquid that solidifies upon cooling.

Gain of moisture

After procedure B: 15 %

Melting point

Before exposure to heat and humidity:	156 °C
After procedure B:	154 °C

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in methanol - water (9:1).

Solvent system:

n-butanol	6 volumes
acetic acid	2 volumes
water	2 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is left in a chromatographic chamber containing some iodine crystals until spots appear.

Results: The spots obtained from the solution of the sample subjected to procedure A and the spots obtained from the reference solution of procaine hydrochloride display the same chromatographic characteristics of migration and detection. After procedure B, tailing is observed below the main spot.

Conclusion

Procaine hydrochloride is subject to slight decomposition under accelerated degradation conditions.

PROCARBAZINE HYDROCHLORIDE

Accelerated Degradation Experiment

Conditions

Procedure A1: 10 days exposure at 50 °C and 100 % relative humidity.

Procedure A2: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 7 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a white to yellowish, crystalline powder, changes after procedures A1, A2 and B into an increasingly yellow-coloured powder and becoming more and more deliquescent.

Gain of moisture

Before exposure to heat and humidity:	1.8 %
After procedure A1:	15 %
After procedure A2:	32 %
After procedure B:	60 %

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in methanol.

Solvent system: concentrated ammonia ..... 1.5 volumes
methanol ..... 100 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is examined under ultraviolet light at a wavelength of maximum output at about 254 nm.

Results: The spots obtained from the solution of the sample subjected to procedure A1 compared to the spots obtained from the reference solution of procarbazine hydrochloride are more numerous and their intensities are increased after procedures A2 and B.

Ultraviolet spectrophotometry

Absorbances measured at about 232 nm of a solution in ethanol 95 %.

Before exposure to heat and humidity:	490
After procedure A1:	460
After procedure A2:	317
After procedure B:	264

A second maximum at about 314 nm appears after procedure A1, the absorbance of which increases after procedures A2 and B.

Potentiometric determination

An acidimetric determination in aqueous solution shows a significant decrease of the content from 94 to 55 % after procedures A1 and B.

Colorimetric determination

To aqueous solutions of the samples subjected to procedures A1 and B, add pyrocatechol in acetone, followed by silver oxide. The absorbance is measured at about 510 nm.

A decrease in absorbance is observed showing a degradation ranging from 6 to 46 %.

Solubility

The substance, originally freely soluble in water, soluble in methanol and ethanol 95 % shows a decrease in solubility after procedures A1, A2 and B.

Conclusion

Procarbazine hydrochloride is subject to strong decomposition under accelerated degradation conditions.

PROMAZINE HYDROCHLORIDE

Accelerated Degradation Experiment

Conditions

Procedure A1: 10 days exposure at 50 °C and 100 % relative humidity.

Procedure A2: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 2 days at 70 °C and 100 % relative humidity.

Procedure C: 3 days exposure at 100 °C in a dry atmosphere.

Appearance of the substance

The substance, originally in the form of a white to yellowish, crystalline powder, changes after procedure A1 into a greenish liquid. No change is observed after procedure C.

Gain of moisture

After procedure A1: 20 %
After procedure A2: 27 %
After procedure B: 30 %

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in methanol.

Solvent system: methanol ..... 1 volume
acetone ..... 1 volume
triethanolamine ... 0.03 volume

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is examined under ultraviolet light at a wavelength of maximum output at about 254 nm, and then sprayed with iodoplatinate reagent (a mixture of 5 ml of a 5 % solution of hexachloroplatinate in 1 N hydrochloric acid, with 45 ml of a 10 % solution of potassium iodide, and 100 ml of water).

Results: The spots obtained from the solutions of each of the samples subjected to procedures A1, A2, B and C, and the spots obtained from the reference solution of promazine hydrochloride display the same chromatographic characteristics of migration and detection.

Ultraviolet spectrophotometry

The absorbance is measured at about 310 nm using a 0.0005 % solution of the samples subjected to the test in 0.01 N hydrochloric acid.

After procedure A2: 68 %. Taking the content of moisture of 27 % into consideration, it represents a degradation of slightly less than 10 %.
After procedure C: no degradation is observed.

Conclusion

Promazine hydrochloride is subject to decomposition after procedures A and B.

PROMETHAZINE HYDROCHLORIDE

Accelerated Degradation Experiment

Conditions

Procedure A1: 10 days exposure at 50 °C and 100 % relative humidity.

Procedure A2: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 4 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a white or faintly yellowish, crystalline powder, changes after procedure A1 into a dark blue, viscous solution, and after procedures A2 and B the colour is intensified.

Gain of moisture

After procedures A1, A2 and B: 55 %

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in ethanol 95 %.

Solvent system: concentrated ammonia ..... 1.5 volumes
methanol ..... 100 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is left in a chromatographic chamber containing some iodine crystals until spots appear.

Results: The spots obtained from the solution of the sample subjected to procedure A1 and the spots obtained from the reference solution of promethazine hydrochloride display the same chromatographic characteristics of migration and detection. Beside the main spot a more mobile spot is observed. After procedure A2, two secondary spots are obtained, one is more mobile than the main spot. The degradation was assessed at less than 5 %. After procedure B, the intensity of the two secondary spots was increased. The degradation was assessed at about 5 %.

Conclusion

Promethazine hydrochloride is subject to decomposition under accelerated degradation conditions. The blue colour assumed by the substance is a very sensitive indicator to test for gross degradation.

PYRIDOXINE HYDROCHLORIDE

Accelerated Degradation Experiment

Conditions

Procedure A: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 4 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of colourless crystals or a white, crystalline powder, changes after procedure A into a humid, light greyish yellow mass. After procedure B two phases appear, an upper clear liquid phase, orange-red in colour, and a lower solid phase of clear crystals.

Gain of moisture

After procedures A and B: 50 %

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in water.

Solvent system:

chloroform	90 volumes
methanol	20 volumes
concentrated ammonia	2 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is left in a chromatographic chamber containing some iodine crystals until spots appear.

Results: The spot obtained from the solution of the sample subjected to procedure A and the spot obtained from the reference solution of pyridoxine hydrochloride display the same chromatographic characteristics of migration and detection. No secondary spots are revealed. After procedure B, the chromatogram performed on the upper liquid phase shows tailing and a less mobile secondary spot behind the main spot. No secondary spots were obtained from the solid phase.

Conclusion

No decomposition is observed by thin-layer chromatography after procedure A, but after procedure B the substance changes as seen from its appearance, gain of moisture and the thin-layer chromatography.

QUININE BISULFATE

Accelerated Degradation Experiment

Conditions

Procedure A : 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B : 30 days exposure at 50 °C and 100 % relative humidity followed by 5 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of colourless crystals or a white, crystalline powder changes after procedure A into pale yellow, crystalline agglomerates, the colour turning to yellow-brown after procedure B.

Gain of moisture

After procedure A : 30 %
After procedure B : 50 %

Chromatographic test

Adsorbent : Silica gel 60 F-254

Solution applied to the plate : Substance dissolved in methanol.

Solvent system : ethyl acetate.....40 volumes
acetone.....38 volumes
n-butanol.....24 volumes
ammonia 10 %..... 8 volumes

Development condition : The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection : The plate is examined under ultraviolet light at a wavelength of maximum output at about 254 nm and then at 365 nm. After spraying with a solution of potassium iodobismuthate the plate is examined in daylight.

Results : The reference solution of quinine bisulfate shows numerous spots of different intensities. After procedures A and B the intensity of some spots increase and additional secondary spots appear.

Ultraviolet spectrophotometry

No modification is observed in the spectrum or in the absorbances at the three maxima of solutions in methanol of the samples subjected to procedures A and B.

Gas-liquid chromatography

After procedures A and B: no change is observed.

Conclusion

Quinine bisulfate is subject to decomposition under accelerated degradation conditions.

QUININE DIHYDROCHLORIDE

Accelerated Degradation Experiment

Conditions

Procedure A : 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B : 30 days exposure at 50 °C and 100 % relative humidity followed by 5 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a white or almost white, crystalline powder changes after procedure A into a yellow-brown, homogenous liquid, the colour turning to brown after procedure B.

Gain of moisture

After procedure A : 65 %
After procedure B : a loss of weight occurs.

Chromatographic test

Adsorbent : Silica gel 60 F-254

Solution applied to the plate : Substance dissolved in methanol.

Solvent system : ethyl acetate.....40 volumes
acetone.....38 volumes
n-butanol.....24 volumes
ammonia 10 %..... 8 volumes

Development conditions : The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection : The plate is examined under ultraviolet light at a wavelength of maximum output at about 254 nm and then at 365 nm. After spraying with a solution of potassium iodobismuthate the plate is examined in daylight.

Results : The reference solution of quinine dihydrochloride shows numerous spots of different intensities. No change is observed after procedure A, but two additional weak spots appear after procedure B.

Ultraviolet spectrophotometry

No modification is observed in the spectrum or in the absorbances at the three maxima of solutions in methanol of the samples subjected to the test.

Gas-liquid chromatography

After procedures A and B: no change is observed.

Conclusion

Quinine dihydrochloride is subject to decomposition under accelerated degradation conditions.

RETINOL (VITAMIN A)

Accelerated Degradation Experiment

Conditions

Procedure A: 10 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 20 days exposure at 50 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of bright yellow flakes, changes after procedure A into an orange-coloured paste, turning to orange-brown after procedure B.

Gain of moisture

Before exposure to heat and humidity:	0.2 %
After procedure A:	5 %
After procedure B:	7 %

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in methanol.

Solvent system: cyclohexane ..... 2 volumes
ether ..... 2 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is examined under ultraviolet light at a wavelength of maximum output at about 254 nm, and then left in a chromatographic chamber containing some iodine crystals until spots appear.

Results: The reference solution of retinol shows a main spot at Rf 0.12, whereas the solution of the sample subjected to procedure A shows several secondary spots, and the main spot represents only about 10 % of the original substance. After procedure B complete degradation is observed.

Ultraviolet spectrophotometry

The absorption spectrum shows that the maximum at 325 nm obtained with the reference substance disappears completely after procedures A and B.

Conclusion

Retinol is subject to decomposition under accelerated degradation conditions.

SALBUTAMOL SULFATE

Accelerated Degradation Experiment

Conditions

Procedure A1: 10 days exposure at 50 °C and 100 % relative humidity.

Procedure A2: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 7 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a white or almost white powder remains unchanged after procedure A2, but changes to a rose-orange coloured liquid after procedure B.

Gain of moisture

Before exposure to heat and humidity:	0.5 %
After procedure A2:	2 %
After procedure B:	80 %

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in methanol.

Solvent system:

concentrated ammonia	4 volumes
water	16 volumes
2-propanol	30 volumes
ethyl acetate	50 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is examined under ultraviolet light at a wavelength of maximum output at about 254 nm, and then left in a chromatographic chamber containing some iodine crystals until spots appear.

Results: The spots obtained from the solutions of each of the samples subjected to procedures A1 and A2, and the spot obtained from the reference solution of salbutamol sulfate display the same chromatographic characteristics of migration and detection. No secondary spots are revealed. After procedure B, five additional spots are detected.

Conclusion

Salbutamol sulfate is subject to decomposition only at higher temperatures as seen after procedure B.

SENNA LEAF

Accelerated Degradation Experiment

Conditions

Procedure A : 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B : 30 days exposure at 50 °C and 100 % relative humidity followed by 5 days at 70 °C and 100 % relative humidity.

Appearance of the material

The material, originally in the form of green-yellowish to green-brownish, thin and brittle leaflets changes after procedures A and B into flexible leaflets, dark brown in colour and spotted with mould.

Gain of moisture :

Before exposure to head and humidity : about 7 %
After procedures A and B : 50 %

Chromatographic test

Adsorbent : Silica gel 60 F-254

Solution applied to the plate : Solution of the material obtained after extraction with hot ethanol 50 %.

Solvent system : water.....3 volumes
ethyl acetate.....4 volumes
1-propanol.....4 volumes

Development condition : The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection : The plate is examined under ultraviolet light at a wavelength of maximum output at about 254 nm and then at 365 nm. After spraying the plate with a 20 % solution of nitric acid, heating at 110 - 120 °C, cooling and again spraying with a 5 % solution of potassium hydroxide in ethanol 50 %, it is examined in daylight.

Results : The reference solution of senna leaf shows numerous spots of different intensities. Important changes are observed after procedures A and B, some spots disappear and new ones occur.

Gas-liquid chromatography

After procedures A and B, the chromatograms show significant changes in the intensities of the major peaks and the disappearance of some of them.

Conclusion

Senna leaf is subject to decomposition under accelerated degradation conditions.

SILVER NITRATE

Accelerated Degradation Experiment

Conditions

Procedure A1: 10 days exposure at 50 °C and 100 % relative humidity.

Procedure A2: 20 days exposure at 50 °C and 100 % relative humidity.

Procedure A3: 30 days' exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 7 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of colourless or white crystals or white cylindrical rods, changes rapidly to a solution that contains in suspension grey insoluble particles.

Gain of moisture

After procedure A3: 46 %
After procedure B: 50 %

Titrimetric determination

Titration with thiocyanate in the presence of ferric ions.

	% decomposed
After procedure A1	6
After procedure A2	21
After procedure A3	60
After procedure B	80

Conclusion

Silver nitrate is subject to decomposition under accelerated degradation conditions.

SODIUM CALCIUM EDETATE

Accelerated Degradation Experiment

Conditions

Procedure A1: 10 days exposure at 50 °C and 100 % relative humidity.

Procedure A2: 20 days exposure at 50 °C and 100 % relative humidity.

Procedure A3: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 5 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a white or creamy white powder, changes after procedures A1, A2 and A3, into a turbid yellowish liquid that contains a white deposit. After procedure B a yellow turbid liquid with a very slight deposit can be observed.

Gain of moisture

Before exposure to heat and humidity:	16 %
After procedure A3:	69 %
After procedure B:	63.5 %

Chromatographic test

Adsorbent: Silica gel G, thickness 0.25 mm.

Solution applied to the plate: Substance dissolved in water.

Solvent system: ethanol 95 %..... 6 volumes
water ..... 2 volumes
concentrated ammonia ..... 2 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: After drying the plate at 70 °C, it is left in a chromatographic chamber containing some iodine crystals until spots appear.

Results: Only one spot is obtained from the reference solution of sodium calcium edetate, whereas from the solution of the samples subjected to procedure A2 a secondary more mobile spot is revealed.

pH value

Prepare a 5 % solution of the substance in water.

Before exposure to heat and humidity:	7.1
After procedure A1:	6.67
After procedure A2:	6.37
After procedure A3:	6.22
After procedure B:	5.95

Conclusion

Sodium calcium edetate is subject to decomposition under accelerated degradation conditions.

SODIUM LACTATE

Accelerated Degradation Experiment

Conditions

Procedure A: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 7 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The material, originally in the form of a transparent, colourless liquid, remains unchanged after procedures A and B.

Gain of moisture

Before exposure to heat and humidity:	39 %
After procedure A:	60 %
After procedure B:	86 %

Chromatographic test

Adsorbent: Silica gel G

Solution applied to the plate: Substance dissolved in ethanol 95 %.

Solvent system: n-butanol ..... 6 volumes
acetic acid ..... 2 volumes
water ..... 2 volumes

Development conditions: The solvent is allowed to migrate until the front reaches 15 cm from the starting line.

Detection: The plate is left in a chromatographic chamber containing some iodine crystals until spots appear.

Results: The spots obtained from the solutions of each of the samples subjected to procedures A and B and the spot obtained from the reference solution of sodium lactate display the same chromatographic characteristics of migration and detection.

pH value

Prepare a 2 % solution of the substance in water.

Indicator	Before exposure to heat and humidity	After procedure A
phenol red	yellow	red-orange
bromothymol blue	green	blue

Conclusion

Sodium lactate is subject to decomposition under accelerated degradation conditions.

SODIUM NITRITE

Accelerated Degradation Experiment

Conditions

Procedure A: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 7 days at 70 °C and a 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a white to slighty yellow, granular powder or white or almost white, opaque, fused masses or sticks, changes after procedures A and B into a colourless to very light yellow liquid.

Gain of moisture

After procedure A: 60 %

Colorimetric determination

Diazo-reaction between solution of the test substance and sulfanilic acid, followed by coupling with 1-naphthylamine; absorbance measured at 520 nm.

After procedure A: no degradation
After procedure B: 25 % degraded

Conclusion

Sodium nitrite is subject to decomposition under accelerated degradation conditions.

SODIUM PARA-AMINOSALICYLATE

Accelerated Degradation Experiment

Conditions

Procedure A: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 5 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a white or lightly coloured, crystalline powder, changes after procedure A into a greyish powder and after procedure B into a black powder.

Gain of moisture

After procedure B: 10 %

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in methanol.

Solvent system: n-butanol ..... 6 volumes
acetic acid ..... 2 volumes
water ..... 2 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is left in a chromatographic chamber containing some iodine crystals until spots appear.

Results: The spot obtained from the solution of the sample subjected to procedure A and the spot obtained from the reference solution of sodium para-aminosalicylate display the same chromatographic charateristics of migration and detection. After procedure B, a large spot with little mobility is obtained, whereas the main spot corresponding to sodium para-aminosalicylate practically disappeares.

Conclusion

Sodium para-aminosalicylate is subject to decomposition under accelerated degradation conditions.

SODIUM STIBOGLUCONATE

Conditions

Procedure A : 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B : 30 days exposure at 50 °C and 100 % relative humidity followed by 4 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a colourless to pale yellow, mostly amorphous powder remains unchanged after procedure A, but is transformed into a colourless and viscous liquid after procedure B.

Gain of moisture

After procedure A : 40 %
After procedure B : 45 %

Chromatographic tests

No migration of the spots could be obtained on silica gel, cellulose, kieselguhr or mixtures of them using 12 different solvent mixtures and 6 different detection methods.

Potentiometric titration

A suspension of the substance containing 0.5 g in 40 ml of water, and titrated with 0.1 N sodium hydroxide shows no change after procedures A and B.

Colorimetric determination

The reaction with the substance is based on the formation of a complex between antimony and iodine ion, absorbance measured at 425 nm. No change is observed after procedures A and B.

Solubility in an aqueous solution

Quantities of 0.05 g of the substance were added to 150 ml of cold water.

Before exposure to heat and humidity:	insoluble
After procedure A:	insoluble
After procedure B:	soluble

Conclusion

Sodium stibogluconate is subject to decomposition under accelerated degradation conditions.

SULFACETAMIDE SODIUM

Accelerated Degradation Experiment

Conditions

Procedure A: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 5 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a white or yellowish white, crystalline powder, changes after procedure A into a viscous, orange-coloured solution and remains similar after procedure B.

Gain of moisture

After procedures A and B: 52 %

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in water.

Solvent system: n-butanol ..... 50 volumes
ethanol 95 %..... 25 volumes
concentrated ammonia ..... 10 volumes
water ..... 25 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is left in a chromatographic chamber containing some iodine crystals until spots appear.

Results: The spots obtained from the solutions of each of the samples subjected to procedures A and B and the spots obtained from the reference solution of sulfacetamide sodium display the same chromatographic characteristics of migration and detection. No secondary spots are revealed.

Conclusion

No decomposition is detected by thin-layer chromatography, but the appearance of the substance changes after procedures A and B.

SULFADIAZINE SODIUM

Accelerated Degradation Experiment

Conditions

Procedure A1: 10 days exposure at 50 °C and 100 % relative humidity.

Procedure A2: 20 days exposure at 50 °C and 100 % relative humidity.

Procedure A3: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 5 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a white or yellowish white powder, changes after procedure A1 into a yellowish pasty mass, after procedure A2 into a yellow liquid paste and after procedure A3 into a liquid.

Gain of moisture

Before exposure to heat and humidity:	3.5 %
After procedure A1:	34.5 %
After procedure A2:	40 %
After procedure A3:	60 %

Solubility

Solution in water.

Before exposure to heat and humidity: clear.
Samples subjected to the test: turbid

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in water.

Solvent system:

n-butanol	50 volumes
ethanol 95 %	25 volumes
concentrated ammonia	10 volumes
water	25 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is examined under ultraviolet light at a wavelength of maximum output at about 254 nm, and then dried and sprayed with a 1 % solution of p-dimethylaminobenzaldehyde in ethanol 95 % containing 1 % of concentrated hydrochloric acid.

Results: The spots obtained from the solutions of each of the samples subjected to procedures A1, A2, A3 and B, and the spot obtained from the reference solution of sulfadiazine sodium display the same chromatographic characteristics of migration and detection.

Ultraviolet spectrophotometry

No change is observed in the spectra of the substance subjected to procedures A1, A2, A3 and B dissolved in ethanolic 0.1 N hydrochloric acid.

Diazo-reaction

No change is observed in the absorbances.

Conclusion

Sulfadiazine sodium is subject to decomposition due to the gain of moisture and the formation of sulfadiazine. The reaction is formed through the absorption of carbon dioxide in air, whereas the structure of sulfadiazine itself remains unchanged.

SULFADIMIDINE SODIUM

Accelerated Degradation Experiment

Conditions

Procedure A: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 7 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a white or creamy white, crystalline powder, changes into a yellow paste, then into a yellow liquid after procedures A and B.

Gain of moisture

Before exposure to heat and humidity:	8 %
After procedure A:	30 %
After procedure B:	45 %

Solubility

Sulfadimidine sodium is very soluble in water and soluble in methanol. After procedures A and B, the solubility is greatly decreased (formation of sulfadimidine).

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in water.

Solvent system:

n-butanol	50 volumes
ethanol 95 %	25 volumes
concentrated ammonia	10 volumes
water	25 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is examined under ultraviolet light at a wavelength of maximum output at about 254 nm, and then sprayed with a 0.1 % solution of 4-dimethylaminobenzaldehyde in ethanol 95 % containing 1 % of concentrated hydrochloric acid.

Results: The spots obtained from the solutions of each of the samples subjected to procedures A and B, and the spot obtained from the reference solution of sulfadimidine sodium display the same chromatographic characteristics of migration and detection. No secondary spots are revealed.

Ultraviolet spectrophotometry

The absorbances measured at about 270 nm remain practically the same for the samples subjected to procedures A and B, as for the reference product and taking the gain of moisture into consideration.

Colorimetric determination

The method is based on a diazo-reaction with nitrite ion in acidic medium, the excess reagent being destroyed with sulfamic acid, and then coupled with N-naphthylethylenediamine. The absorbance is measured at about 535 nm.

The absorbances for the samples under test remain practically unchanged.

Conclusion

No decomposition is detected by TLC, by UV or colorimetric determination, but the gain of moisture and the change in solubility show that the substance is subject to decomposition under accelerated degradation conditions.

SUXAMETHONIUM CHLORIDE

Accelerated Degradation Experiment

Conditions

Procedure A: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 7 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a white or almost white, crystalline powder, changes after procedure A into a white, humid mass and after procedure B into two phases, an upper clear liquid one, and a lower one as a white, solid mass.

Gain of moisture

Before exposure to heat and humidity:	9 %
After procedures A and B:	40 %

Chromatographic test

Adsorbent: Cellulose

Solution applied to the plate: Substance dissolved in methanol.

Solvent system:

2-propanol	6 volumes
water	3 volumes
acetic acid	1 volume

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is left in a chromatographic chamber containing some iodine crystals until spots appear.

Results: The spots obtained from the solution of the sample subjected to procedure A and the spot obtained from the reference solution of suxamethonium chloride display the same chromatographic characteristics of migration and detection. After procedure B, an additional spot is observed which is more mobile than the main spot.

Conclusion

Suxamethonium chloride is subject to decomposition only at higher temperatures as seen after procedure B.

TETRACAINE HYDROCHLORIDE

Accelerated Degradation Experiment

Conditions

Procedure A1: 10 days exposure at 50 °C and 100 % relative humidity.

Procedure A2: 20 days exposure at 50 °C and 100 % relative humidity.

Procedure A3: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 7 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a white, crystalline powder, changes after procedures A1, A2, A3 and B into a viscous, slightly chestnut coloured liquid. After cooling, solidification occurs and the substance appears as fine, white crystals.

Gain of moisture

Before exposure to heat and humidity: 0.2 %
After procedures A3 and B: 35 %

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in methanol.

Solvent system: methanol ..... 100 volumes
concentrated ammonia ..... 1.5 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is examined under ultraviolet light at a wavelength of maximum output at about 254 nm, and then left in a chromatographic chamber containing some iodine crystals until spots appear.

Results: The spots obtained from the solutions of each of the samples subjected to procedures A1, A2, A3 and B, and the spot obtained from the reference solution of tetracaine hydrochloride display the same chromatographic characteristics of migration and detection. No secondary spots are revealed.

Ultraviolet spectrophotometry

The spectrum recorded between 200 nm and 350 nm, using 0.001 % solutions of the samples subjected to procedures A3 and B in water, show an absorption maximum at about 311 nm. No degradation is observed.

Non-aqueous titration

The titrations with 0.1 N perchloric acid of 70 mg of each of the samples subjected to procedures A3 and B, dissolved in glacial acetic acid with the addition of mercuric acetate, show no degradation.

Colorimetric determination

To an aqueous solution of the samples subjected to the test, at pH 2, add picric acid, and extract the yellow picrate into chloroform. Absorbance of the organic layer measured at 410 nm: no degradation is observed.

Conclusion

No decomposition is detected by TLC, UV, the colorimetric determination and the non-aqueous titration, but the appearance of the substance changes after procedures A and B.

TETRACYCLINE HYDROCHLORIDE

Accelerated Degradation Experiment

Conditions

Procedure A: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 5 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a yellow, crystalline powder changes after procedure A into a dark yellow powder and after procedure B into a dark brown powder.

Gain of moisture

After procedures A and B: 15 %

Chromatographic test

Adsorbent: Cellulose with a fluorescent indicator, 0.1 mm in thickness, impregnated with 0.1 M disodium edetate and dried at 100 °C for 30 minutes.

Solution applied to the plate: Substance dissolved in methanol. After application the plate is exposed to ammonia vapours for 15 minutes.

Solvent system:

n-butanol	10 volumes
pyridine	6 volumes
ethyl acetate	6 volumes
water	7 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is exposed to ammonia vapours and is examined under ultraviolet light at a wavelength of maximum output at about 365 nm.

Results: The secondary spots obtained from the solution of the sample subjected to procedure A compared to the spots obtained from the reference solution of tetracycline hydrochloride are more intense. The secondary spots correspond to anhydrotetracycline and to epianhydrotetracycline. After procedure B, the secondary spots are intensified indicating gross degradation.

Conclusion

Tetracycline hydrochloride is subject to decomposition under accelerated degradation conditions.

THIAMINE HYDROCHLORIDE

Accelerated Degradation Experiment

Conditions

Procedure A: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 4 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of colourless crystals or a white or yellowish white, crystalline powder with a slight and characteristic odour acquires gradually after procedures A and B a more pronounced colour and odour. It changes into a colourless to yellowish solution after procedure A and becomes bright yellow after procedure B.

Gain of moisture

After procedures A and B: 168 %

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in water.

Solvent system:

n-butanol	50 volumes
ethanol 95 %	25 volumes
concentrated ammonia	10 volumes
water	25 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is left in a chromatographic chamber containing some iodine crystals until spots appear.

Results: The spots obtained from the solutions of each of the samples subjected to procedures A and B and the spots obtained from the reference solution of thiamine hydrochloride display the same chromatographic characteristics of migration and detection. No secondary spots are revealed, but the main spot decreases in intensity.

Conclusion

No decomposition is detected by thin-layer chromatography, but the appearance of the substance changes after procedures A and B.

THIAMINE MONONITRATE

Accelerated Degradation Experiment

Conditions

Procedure A: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 7 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of colourless crystals or a white, crystalline powder, shows no significant modification after procedure A, but changes into a yellow liquid after procedure B.

Gain of moisture

Before exposure to heat and humidity:	none
After procedure A:	2 %
After procedure B:	40 %

Solubility

Dissolve 1 g in 50 ml of water. The solubility does not change after procedure A, but decreases after procedure B.

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in ethanol 95 % at a temperature of 50 °C.

Solvent system:

n-butanol	50 volumes
ethanol 95 %	25 volumes
concentrated ammonia	10 volumes
water	25 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is examined under ultraviolet light at a wavelength of maximum output at about 254 nm.

Results: The spot obtained from the solution of the sample subjected to procedure A and the spot obtained from the reference solution of thiamine mononitrate display the same chromatographic characteristics of migration and detection. After procedure B several secondary spots are revealed.

Ultraviolet spectrophotometry

Before exposure to heat and humidity and after procedure A, the absorbances at 271 nm remain practically the same.

$$A^{1\ \%}_{1\ cm} = 350.$$

The absorbance decreases to 110 after procedure B.

Potentiometric determination

A non-aqueous titration shows no significant degradation.

Conclusion

Thiamine mononitrate is subject to decomposition only at higher temperatures as seen after procedure B.

THIOPENTAL SODIUM

Accelerated Degradation Experiment

Conditions

Procedure A1: 20 days exposure at 50 °C and 100 % relative humidity.

Procedure A2: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 7 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a yellowish white powder, changes after procedures A2 and B into small humid agglomerates.

Gain of moisture

Before exposure to heat and humidity: 6 %
After procedures A2 and B: 30 %

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in methanol.

Solvent system: chloroform ..... 9 volumes
acetone ..... 1 volume

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is examined under ultraviolet light at a wavelength of maximum output at about 254 nm, and then left in a chromatographic chamber containing some iodine crystals until spots appear.

Results: After procedure A1 a secondary spot is observed that is more mobile than the main spot. After procedure B the secondary spots are intensified.

Conclusion

Thiopental sodium is subject to decomposition under accelerated degradation conditions.

TOLBUTAMIDE

Accelerated Degradation Experiment

Conditions

Procedure A: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 4 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a white or almost white, crystalline powder remains unchanged after procedure A, but changes into a solid, pale yellow mass after procedure B.

Gain of moisture

After procedures A and B: 0.7 %

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in chloroform.

Solvent system: cyclohexane ..... 7 volumes
acetone ..... 3 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is left in a chromatographic chamber containing some iodine crystals until spots appear.

Results: The spots obtained from the solution of the sample subjected to procedure A and the spots obtained from the reference solution of tolbutamide display the same chromatographic characteristics of migration and detection. No secondary spots are revealed. After procedure B, two additional spots are revealed that are less mobile than the main spot.

pH value

Prepare an aqueous suspension.

After procedure A: 4.8
After procedure B: 7

Conclusion

Tolbutamide is subject to decomposition under accelerated degradation conditions.

UNDECYLENIC ACID

Accelerated Degradation Experiment

Conditions

Procedure A: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 5 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a white, crystalline mass at temperatures below 25 °C and a colourless liquid at temperatures above 25 °C, acquires after procedure A a pale yellow colour and after procedure B an orange colour.

Gain of moisture

None after procedures A and B.

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in methanol.

Solvent system:

chloroform	90 volumes
acetone	10 volumes
acetic acid	5 volumes

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is left in chromatographic chamber containing some iodine crystals until spots appear.

Results: After procedure A, additional spots were revealed. The degradation was estimated at 2 %. After procedure B, the degradation was estimated at about 5 %.

Conclusion

Undecylenic acid is subject to decomposition under accelerated degradation conditions.

WARFARIN SODIUM

Accelerated Degradation Experiment

Conditions

Procedure A1: 10 days exposure at 50 °C and 100 % relative humidity.

Procedure A2: 20 days exposure at 50 °C and 100 % relative humidity.

Procedure A3: 30 days exposure at 50 °C and 100 % relative humidity.

Procedure B: 30 days exposure at 50 °C and 100 % relative humidity followed by 7 days at 70 °C and 100 % relative humidity.

Appearance of the substance

The substance, originally in the form of a white, amorphous or crystalline powder, changes after procedures A1, A2, A3 and B into a liquid that contains an insoluble white matter.

Gain of moisture

Before exposure to heat and humidity:	0.42 %
After procedure A3:	35 %
After procedure B:	39 %

Chromatographic test

Adsorbent: Silica gel F-254

Solution applied to the plate: Substance dissolved in ethanol 95 %.

Solvent system: chloroform ..... 90 volumes
methanol ..... 20 volumes
concentrated ammonia ..... 1 volume

Development conditions: The solvent is allowed to migrate until the front reaches a line 15 cm from the starting line.

Detection: The plate is examined under ultraviolet light at a wavelength of maximum output at about 254 nm, and then left in a chromatographic chamber containing some iodine crystals until spots appear.

Results: After procedures A and B, three additional spots are detected and the main spot is less intense than the spot corresponding to the reference solution of warfarin sodium.

Ultraviolet spectrophotometry

Prepare a solution containing 1.0 mg of the substance in 100 ml of ethanol 95 %.

	Absorbance at 308 nm
Before exposure to heat and humidity	0.375
After procedure A1	0.350
After procedure A2	0.300
After procedure A3	0.288
After procedure B	0.270

pH value

Prepare a solution containing 0.1 g of the substance in 10 ml of water.

Before exposure to heat and humidity: 8.5
After procedure B: 10

Conclusion

Warfarin sodium is subject to decomposition under accelerated degradation conditions.

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